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MEDICAL TECHNOLOGY AND BIOLOGICAL RESEARCH*

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As the number of medical technologists increases and their field of endeavor broadens, it is inevitable that specialization should begin to appear. Besides the technologists who spend their time in hospital laboratories and doctors' offices, we have specialists in bacteriology, chemistry, and blood banking, and it is now proposed to include cytology and radioisotope technicians as well. Some technologists have found their way into research laboratories in medical schools, in government agencies and, a few, in laboratories connected with industry.

This last group is small. In general, little is known of their activities, but there seems to be a tendency to regard them with suspicion, as though in becoming allied with industry such people had turned their backs on suffering humanity in favor of easy jobs, shorter hours, and higher wages. This is not necessarily the case. Those of us who have chosen this field feel that our work is just as important as caring for the sick and certainly every bit as interesting. In some ways it is preventive research, for the objective is the development of new tools for the treatment or prevention of disease as well as protection of the public health by a thorough study of the safety or potential hazard of all new products intended for consumption by the unwary public. These products include all imaginable kinds of things—additives for foods, to preserve, flavor or color them; drugs; dyes for many purposes; cosmetics; antiseptics; soaps and detergents; lubricants for automobiles and other machinery; compounds for use in paper; agricultural chemicals, insecticides and rodenticides; and a vast array of industrial chemicals and materials which appear as intermediates in many manufacturing processes.

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The necessity and importance of research and testing laboratories are obvious when we remember the Salk vaccine scare of last year. A few years before that considerable newspaper publicity was given to the deaths caused by the consumption of an elixir of sulfanilamide. Boric acid, an old familiar drug, also made the headlines when it was revealed as the cause of death of babies in whose formula it had accidentally been substituted for malt sugar. An artificial flavoring, widely used in food manufacturing and considered quite safe because it was from natural origin, was suddenly withdrawn from the market when it proved responsible for severe damage to the livers and kidneys of experimental dogs receiving this material in their daily diet over a period of time. What, if anything, it had been doing to human livers and kidneys we do not know. Similarly, three dyes which had been certified by the Food and Drug Administration as safe for use in foods, drugs and cosmetics were found to cause cirrhotic changes in the livers of rats on chronic feeding studies.

These incidents are shocking and fortunately very rare, but few people are aware of the efforts which must be made to avoid them. We take for granted the safety of our food, cosmetics, clothing, and almost everything we use which is not actually labeled with skull and crossbones. We believe that if we follow the directions on a bottle of medicine it will cure our ailments without harming us in some other way. Those of us who are not employed in chemical plants or on farms are not conscious of the need for an understanding of the nature and physical effects of various industrial and agricultural agents. Yet casual contact with compounds of which nothing is known may result in sickness, permanent injury, or death.

There are laws and regulations controlling the toxicity or hazard of new materials appearing on the market. The first federal food and drug law was drafted in 1906. A major revision was passed in 1938 following public reaction to the elixir of sulfanilamide episode, and new amendments have continually been proposed. Newspapers and magazines have helped direct public attention to the need for legislation by publicizing occasions such as those mentioned above. The reputable producer is guided not only by the law but by the expedience of knowing all that he can about his product before putting it on the market for sale. He is also bound by law to provide adequate protection for his employees against any toxic materials used in or resulting from his manufacturing processes.

Safety screening begins with laboratory animals. The old philosophy of "trying it on the dog" is extended to include mice, rats, rabbits, guinea pigs, cats, and sometimes hamsters, monkeys, chickens, geese, turkeys, pigs, sheep and cows as well as dogs. The first step is usually to establish an LD_{50}. This term

indicates the dose of the compound which will kill 50 per cent of the animals receiving it and is determined statistically by one of several methods from the experimental data. Depending on the intended use of the compound, any of several modes of administration will be employed. Materials for cosmetic use will be tested dermally by single or repeated application to the shaved skin of rabbits. If no local irritation or systemic toxicity is observed, a human subject, having far less sensitive skin, can feel safe in applying the material to his own body. Eye irritation studies, again using the rabbit, are done when there is a possibility of the material entering the eyes, either intentionally or accidentally, as can happen with cold wave lotions used by an individual at home. Some compounds are given intraperitoneally or intravenously. Perhaps the most frequent mode of administration is oral; this method seems to be used for good measure on nearly all materials under study because, as one worker put it, "there is no predicting what some people will swallow." Vapors and dusts, which may be inhaled, are tested by exposing animals to them in inhalation chambers.

After an acute LD_{50} is established, chronic feeding studies may follow. Here dogs and rats are used because of the similarity of their metabolism to that of human beings. The study may run for 30 days or as long as two years with the animals on a standard laboratory diet containing the compound. Weekly records are kept of body weight and food consumption.

The large economic animals are generally reserved for studies of agricultural chemicals and veterinary medicines, although young pigs are valuable for testing materials to be used on the skin. Now and then a study will end in human subjects. This comes only after sufficient evidence of its safety has been compiled in lower animals. The laboratory workers themselves frequently volunteer for this phase.

Along with animal testing, especially in chronic feeding studies, various clinical procedures are used. Sometimes special modifications in technic or equipment are necessary. Blood counts can be made from venous blood in rabbits and larger animals. With rats and mice, blood must be collected from the tip of the tail, unless the animals are due for sacrificing and heart puncture may be done. Routine urinalyses are performed at intervals on individual dogs and on pooled specimens from groups of rats receiving identical treatment. Blood chemical determinations are also done, both to estimate the degree of damage produced in various organs by the test material and to find out the concentration of the material in the body.

Radiographic media to aid in visualization of many different parts of the body are screened for safety by means of intravenous injections in rats before they are released for injection

into humans. They are studied also by X-ray in actual use in animals to see how good a job they do. Bacteriological procedures are adapted for various purposes and sometimes bear little resemblance to the diagnostic bacteriology of a hospital laboratory. An example of this is in antiseptic testing where stock strains of known bacteria are tested against compounds of unknown germicidal activity. Histology provides another useful tool. At termination of chronic studies the animals are sacrificed and autopsied. Tissues from representative animals are preserved in formalin, and slides are prepared and read by a pathologist. Thus additional information to that found in the weekly records of weight gain and food consumption and in the gross autopsy is obtained.

When new drugs and some extremely toxic compounds, such as the insecticides, are studied, investigation is often made into the mechanism of their action. If these materials are sufficiently superior to the older ones which they are intended to replace, so that there is a possibility that they will be marketed, it is very important that the nature of their toxicity be known so that antidotes can be sought. This involves some of the most interesting research of all. Guinea pig tracheal chain tests, isolated rabbit heart technics, blood pressure and respiration studies, encephalography—these and other tests are used in pharmacodynamics, where a drug or material of unknown action is pitted against one of known effect in an effort to determine its mode of action.

In order to understand variations in animals undergoing experimentation, a knowledge of normal values is essential. The experimental worker is often obliged to supply this information himself. This is one reason for the group of control animals which are always included in an experiment—to supply a comparative "normal."

Hematology in animals proves to be quite a surprise to the uninitiated. Kracke, quoting from Scarborough's monograph, gives ranges of normal leukocyte counts which are far wider than for human counts. For the rabbit "normal" is 4,000-13,000; for the dog, 6,000-20,000; for chickens, 20,000-40,000. Since experimental dogs are usually screened before being used and only healthy ones considered acceptable, it is a question whether or not to regard an animal with a leukocyte count of 20,000 as normal. In our laboratory, the best results have been obtained with those showing a count not higher than 10,000. Most laboratory animals show a higher red cell count than is found in humans. Since the normal rat erythrocyte count is 7,000,000-10,000,000, it has been the practice in our laboratory to fill the pipette with only three-fifths of the usual amount of blood with the standard pipette. It is felt that the errors inherent in using

this greater dilution of blood are no more than those involved in trying to count the higher number of cells in the hemacytometer. There are available both leukocyte and erythrocyte pipettes which require one-third of the usual amount of blood in the standard diulution.

Interestingly, Scarborough gives no average or range for the leukocyte count in rats. We have found it to run about the same as in dogs; that is, 6,000-16,000. The method of collection affects this quite markedly. Our usual method is to clip the tip of the tail and fill our pipettes from the free-flowing blood. On one occasion we decided to use heart blood, as the animals were to be sacrificed. The result was a startling drop in the leukocyte count, to less than 5,000. Apparently the mechanical manipulation of the tail is sufficient to stimulate the higher count. This may leave one wondering about the value of doing blood counts in rats, but it is only by the compilation of data on counts and on the technics of collecting them that any decision can be reached.

Rats are notoriously able to resist infection. But one rat was found to have a leukocyte count of 120,000. In a human patient this would have led to a speedy diagnosis of some type of leukemia. In the rat this was caused by a large abscess and was judged to be a normal response.

Upon first examining a differential smear from a rabbit one is struck by the tremendous number of eosinophiles. After a little experience the distinction between true eosinophiles and the pseudo-eosinophiles, which predominate and correspond to the neutrophiles of humans, is not too hard to see. All the rodents show a reversal of the poly-lymph ratio found in humans. The differential of the dog is similar to that of the human except for eosinophilia, caused by parasitic infestation which is quite common.

In histology virtually the same methods are used as for human organs. Here, however, a more carefully prepared neutral formalin is important in order to prevent distortion of the cells. In reading slides prepared from human material, a pathologist looks for certain patterns caused by particular diseases. Slides from experimental animals can be complicated by variations within normal limits, post-mortem changes, and changes due to parasitic infestation; and distortion caused by improper fixation or dehydration further complicate matters for the pathologist, who must decide what damage has been done by the compound under study. Here again the inclusion of a group of controls is helpful in providing a basis for comparison, except when it happens that the controls show more abnormalities than the test animals.

Some of the methods used originally in research have come into use in the clinical laboratory. Ten years ago penicillin and streptomycin sensitivity tests were so time-consuming that the

busy hospital lab had no time for them. Now, with the introduction of the dry treated discs and rings they have become everyday routine. The Michel test for cholinesterase activity was simply a research method until the widespread use of organic phosphate insecticides on field crops, with its attendant dangers to the farmer using them, made it necessary to provide some means of determining the amount of exposure he had sustained. Similarly, through the research laboratory has come a means of treatment for this type of poisoning. Production of polio vaccine was made possible by use of tissue culture, a technic which also offers possibilities as a method for toxicity studies.

After watching intravenous administration in mice, a Health Department worker was led to try the same technic for pregnancy tests, thus combining the speed of the Friedman test with the economy of the Aschheim-Zondek test. In this era of frog tests, this may not be a matter of great importance, but it is interesting. It might be said here that venipunctures in small animals are not so difficult as one might think. It is much easier than trying to locate a collapsed vein in a fleshy patient.

Research is seldom as exciting or dramatic as the movies and television make it seem. Like most other jobs it quickly falls into routine in its daily aspects. Certainly by the time a medical technologist has finished her training she is accustomed to performing routine tasks well. She has sufficient education in basic science to give her a background for biological research. The manual dexterity acquired in the hospital laboratory, as well as many of the procedures used there, can be used just as easily in the investigational laboratory. Attention to detail, careful observation of the results of her work, and the keeping of accurate records are important in both fields.

Perhaps you are asking yourselves just how a medical technologist can prefer working with animals to working with people, as she was trained to do. The answer to this is individual preference. Not everyone likes this type of work, or is suited to it, but those who go into it usually find it as rewarding as work with patients. It provides a change from hospital routine, to say the least. There is less haste, because in research no one can predict how an experiment will go until it is actually under way. Here, as seldom happens in the clinical laboratory, one gets to see the relationship of medicine to all other branches of biology, chemistry and physics in their broadest aspects. Often there are stimulating contacts with top research people of all fields.

It gives one a sense of looking into the future as most of the materials tested have not yet reached the stage of production and marketing. There is a thrill in doing an LD_{50} on a new material and knowing that you are the first and perhaps will be the only one ever to do this particular test on this particular

compound, and therefore you are responsible for doing it right. There is a sense of satisfaction in seeing so ordinary a thing as a new hand lotion appear in the stores and knowing that it is just as gentle as it claims to be, yet remembering its earliest formulation—so irritating that the skin of the test rabbits became jelly-like with edema and so painful that the slightest handling caused them to scream. This may not be strictly medicine, but it does help us all to safer living.

REFERENCE

Scarborough, R. A.: The Blood Picture of Normal Laboratory Animals, New Haven, Conn., Yale, 1927.

ANNOUNCEMENTS

ANNOUNCING a medical placement exchange program. Dr. Laureane T. Angeles, M.D., Director of Operations, Philippine Medical Placement Program, Inc., Kendall Terrace, Corner Isaac Peral, Nebraska, Manila, Philippines, has written concerning the possibility of sending Philippino trainees to United States hospitals with Approved Schools for the training of medical technologists. Dr. Angeles asks that those hospitals who are interested communicate with him directly. He further states, "Generally we send only pharmacy student graduates, since they meet the requirements of the Registry for medical technologists, and we send them to schools that give free board and lodging and a little stipend for pocket money, although we would prefer it if they could be given a nominal stipend if possible."

We feel as Dr. Angeles does, that this program will be of mutual benefit in promoting good will and closer understanding between the two countries. Please write to Dr. Angeles if you would like to participate.

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The 34th Annual Conference of the American Physical Therapy Association will be held at the Hotel Statler in Detroit, Michigan, June 23-29, 1957.

HEXACHLOROPHENE IN THE SURGICAL SCRUB*

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Since the early appreciation by Lister (1867) of the idea of antiseptic surgery, many improvements in technic and removal of many possible sources of wound contamination have resulted in aseptic surgery. Evaluation of sources of surgical infection in "clean" operations is still a complex problem. Kraissl (1950) considered the air as probably first in importance, but Devenish and Miles (1939) blamed most of their infected wounds on leakage through glove punctures from the hands of a surgeon who was a skin carrier of *Staphylococcus aureus*. The same person may be both a skin and nasal carrier (Gillespie *et al.*, 1939; Landy *et al.*, 1955), and thus a possible double source of infection. The hands of the surgeon and those of persons handling material or instruments which may have contact with the field of operation must be thoroughly "degermed" (Price, 1938) in order to minimize the danger to the patient from skin flora of the surgical personnel. "Degerming" implies killing or removal of bacteria whether or not they are agents of infection.

Transient flora may be abundant at times but are relatively sparse on grossly clean skin (Price, 1938). The predominant residents found on the skin consist of coagulase negative micrococci (Pillsbury *et al.*, 1942). These are difficult to remove and only slowly destroyed. Although these organisms are usually of low pathogenicity (Nungester *et al.*, 1949), the ability of some pathogens, especially the hemolytic staphylococci, to establish themselves as part of the resident flora demands great care in degerming the hands of the operator (Gillespie *et al.*, 1939).

Attempts have been made to sterilize the skin by chemical means, using phenols, cresols, alcohols, organic and inorganic mercurials, quaternary ammonium compounds, halides, detergents, and other compounds. Most methods of preparation of the hands have involved a thorough scrubbing with soap and vigorous brushing followed by a soak in a chemical bath (Blank *et al.*, 1950). One of the most effective of the chemical agents and one which retains a large part of its bactericidal activity in the presence of excessive amounts of soap (Gump, 1945) is a diphenol, hexachlorophene, the designation recognized by the Council on Pharmacy and Chemistry of the American Medical Association (1948) for bis-(2-hydroxy-3,5,6-trichlorophenyl) methane. It is relatively nontoxic when given orally to guinea pigs (Gump, 1945) but highly toxic intravenously in dogs (Price and Bonnett, 1948). Its toxicity upon absorption from intact skin when in

* Hillkowitz Award. Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

organic solvents is not known but it does not produce irritation or sensitivity as shown by numerous patch tests carried out by Traub *et al.* (1944), and Udinsky (1945).

In vitro hexachlorophene has a phenol coefficient by FDA methods against *Micrococcus pyogenes* of 125, but of only 20 against gram negative organisms such as *Salmonella typhi* or *Escherichia coli* (Traub *et al.*, 1944). Studies by various technics (Price and Bonnett, 1948; Reid *et al.*, 1950; Seastone, 1947; Traub *et al.*, 1944) following single uses of hexachlorophene and use over several days either exclusively or in the surgical scrub, have shown the gradual reduction in the resident flora of the hands. In an attempt to define both the concentration of hexachlorophene and the pattern of usage which would give best results, this study of the use and effectiveness for the surgical scrub of commercially available cleansing agents containing hexachlorophene was undertaken.

Methods

The hexachlorophene containing agents intended for surgical use (Table 1) were three soaps, Hexachlorophene Germa Medica,[1] Four Star,[1] and Septisol,[2] and a detergent, pHisoderm (sodium acetylphenoxyethoxyethyl ether sulfonate with lanolin, cholesterol, and mineral oil) with hexachlorophene, now called pHisohex.[3] Vestal Antiseptic Liquid Soap[2] was included in the cumulative series with intermittant use. Where more than one hexachlorophene soap was produced by a single manufacturer the same soap base was used for the products, the only difference being in the hexachlorophene content.

TABLE 1

Hexachlorophene Concentration in Cleansing Agents

Cleansing Agent	Percent Hexachlorophene		Use Dilution	Use Percent of	
	By Weight	By Soap Solids		Hexachlorophene	Soap
pHisohex	3			3	
Four Star	4	10	1 + 3	1	10
Vestal Antiseptic Liquid Soap	1.85	5	1 + 2	0.62	12.5
Germa Medica	1	2.5	1 + 3	0.25	10
Septisol	0.75	2	1 + 2	0.25	12.5

Preliminary examinations were made by swabbing the hands of the operating personnel during and after the standard scrub of ten minutes with one minute soak in 1:1000 aqueous Zephiran[4] and after operations wearing gloves from one to seven hours. The operating room personnel, surgeons, and medical students

[1] Supplied by the Huntington Laboratories, Inc., Huntington, Indiana.
[2] Supplied by the Vestal Laboratories, Inc., St. Louis, Missouri.
[3] Supplied by the Winthrop Laboratories, New York, New York.
[4] Winthrop Laboratories, New York, New York.

were surveyed with single basin scrubs.

For thorough study a series of weekly scrubs for ten months was done on a small group of persons who did not scrub except for purposes of this study. Timed scrubs in a series of basins based on the method of Price (1938) were used. No attempt was made to have brushes of constant stiffness nor to control the vigor with which they were used. As the series continued the bacterial counts from the individuals showed uniformity in their relation to each other which indicated that they were following a consistent pattern in scrubbing. The serial basins allowed the introduction of the test agent in one or more basins (Pohle and Stuart, 1940), made apparent and minimized the effect of carry over of the test agent (Seastone, 1947), made possible comparisons of counts before and after wearing gloves, and made possible comparisons of the effects of scrubbing with or without the test agent.

To test the cumulative effect of the hexachlorophene, the test agent was used exclusively for all washing at the hospital and at home, and subsequently the test agent was used intermittently for four days according to the manufacturer's directions. To the latter series was added a four day period of the standard surgical scrub.

The standard surgical scrub consisted of the use of soft soap solution and tap water rinses for ten minutes, brushing thoroughly all parts of the hands with special attention to the nails, followed by a one minute soak in 1:1000 aqueous Zephiran. Manufacturer's directions were followed during the days of intermittent scrubs for the cumulative series. For pHisohex a 20 second wash with nail cleansing was used followed by rinsing and a scrub of 15 strokes with a brush to all parts of the hands and 25 strokes to the nails. This requires at least two minutes for the two hands, thus including a two minute scrub in the two and one-half minutes of washing. The directions for the use of Four Star and Germa Medica soaps both call for three consecutive one minute washes with brush to the nails for one-half minute. Vestal Antiseptic Liquid Soap and Septisol were used for two two minute washes, not consecutive, and a brush to the nails for one-half minute. The directions for this called for any schedule of washes for a total of three and one-half to six minutes a day.

After the exclusive use of hexachlorophene agents for one week (pHisohex was repeated after one to six months), a three basin scrub using only Ivory soap and water was done. The test agent was reapplied as if for surgery preparation, gloves were worn for two hours, and another three basin Ivory scrub followed.

No gloves were used in the intermittent series since the series of constant use had shown little difference before and after the gloves, but the Ivory soap scrubs were done for five two minute

basins to allow more adequate removal of the transient flora and to stabilize the counts.

The test scrubs were all done in basins containing approximately two liters of water, sterilized water from the operating room supply being used for some and tap water for the others. Approximately twelve ml of well mixed water was taken with a sterile pipette from each basin as the scrub was completed in it. This was transferred to a sterile test tube. For the series in which serum was used to minimize the effect of the hexachlorophene (Fahlberg *et al.*, 1948), one ml of Seitz-filtered inactivated pooled human serum was placed in the tubes before collection from the basin. Serum and non-serum containing tubes were filled at the same time. At the conclusion of each scrub the tubes were taken from the operating room floor to the bacteriology laboratory for plating, with an interval of usually about twenty minutes between the scrub and the addition of agar. Three samples, 0.1, 1.0, and 10 ml, were placed in sterile Petri dishes and melted nutrient agar held at 45 C was added to each plate with immediate thorough mixing by rotation. Double strength agar was used for the plates containing 10 ml of scrub water. The plates were incubated at 37 C for 48 hours before counting. A total count was made on plates with two to three hundred or fewer colonies and fractional counts using the Stewart plate in the Quebec colony counter on those with higher counts. Plate counts above about ten thousand were not attempted and the average for that sample was based on the other dilutions. All counts are reported as the total number of visable organisms present in the basin of scrub water.

Results

Skin swabs.—Swabs of the palms of the hands after two, five, and ten minutes pre-operative scrubbing with Septisol gave only one negative from twenty-five cultures. After a one minute Zephiran soak and a lapse of ten minutes, six negative cultures were obtained from ten swabs. Seven of these persons were swabbed again after from two and a half to seven hours in gloves and five negative cultures were otbained. Nine persons were swabbed immediately after the hands were dried on sterile towels following the standard scrub with Zephiran soak. Of these eight showed negative cultures. Post operatively seven of eight of this group still had negative cultures.

Single basin scrubs.—The control scrubs with water only and with Ivory soap and water each averaged about one million organisms removed from the two hands in approximately two minutes. Counts after a ten minute scrub with soft soap solution showed a drop to about ten per cent of the scrubs without preparation. A Zephiran rinse after the soap scrub decreased the number of viable organisms to about one per cent of the initial count.

A three minute scrub with pHisohex took the count to about the same level as the green soap and Zephiran had done and an additional Zephiran rinse after the pHisohex reduced the average count to about a thousand viable organisms or approximately one-tenth per cent of the number from the unprepared hands.

Serial basin scrubs: Controls and normals.—Control counts made from basins of sterilized water from the operating room supply and of the tap water as they stood in the operating room in readiness for the scrub showed an average of 2,332 organisms per basin in 75 samples of sterilized water and an average of 2,880 organisms per basin in 50 samples of tap water. These counts were so much lower than those obtained in most scrubs that they have been disregarded in considering changes of counts with variations in test agents.

Ideally the counts from each sample should be proportional to the 0.1, 1.0, and 10.0 ml of wash water plated (Table 2). However, the crowding and decreased accuracy in counting in the plates containing the larger volumes of wash water may give them a lower proportion, and the relative number of air and water bacteria in the plates with lower counts may give them a higher proportion than should be realized. Changes from the ratios obtained under control conditions may be indicative of other factors such as carry over of disinfectant from a previous basin or the effect of soap or test agent in the basin being counted. Notwithstanding the deviation of ratios of the plate counts with the Ivory soap, this method was used as a base line

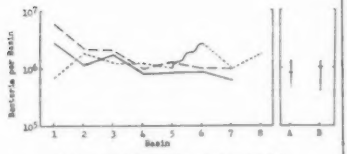
TABLE 2

Ratio of Colony Counts in 0.1 Ml.c., 1.0 Ml.c. and 10 ML Samples of Water From Scrub Basins

Type of Scrub and Number of Basins	SAMPLE SIZE		
	0.1 ml	1.0 ml	10.0 ml
Water (49)	0.113	1.00	11.6
Ivory Soap (106)	0.140	1.00	7.8
Ivory Soap—with serum (7)	0.166	1.00	7.9
—without serum (7)	0.103	1.00	7.3
Soft soap (20)	0.193	1.00	6.4
Ivory after alcohol soak—1st basin (4)	0.122	1.00	6.1
—2nd basin (4)	0.085	1.00	8.5
—3rd basin (4)	0.102	1.00	6.7
Ivory after Zephiran soak—1st basin (5)	0.958	1.00	2.3
—2nd basin (4)	0.091	1.00	10.2
—3rd basin (4)	0.070	1.00	6.7
Ivory after pHisohex basin—1st basin (5)	0.666	1.00	1.8
—2nd basin (5)	0.273	1.00	2.7
—3rd basin (5)	0.522	1.00	4.6
Septisol, serial scrubs (30)	1.10	1.00	1.25
Germa Medica, serial scrubs (30)	1.07	1.00	0.04
Four Star, serial scrubs (30)	6.5	1.00	0.17
pHisohex, serial scrubs (30)	45.8	1.00	1.75

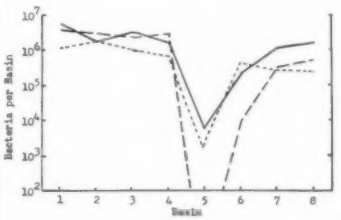
in all test scrubs, since the antibacterial effect of the soap was relatively low and since the soap served as a lubricant in the scrubbing, resulting in less irritation to the hands than when water alone was used.

Plain water and Ivory soap scrubs in the serial basins showed a lower count in successive basin until the count reached about a million, where it stabilized (Fig. 1). A lapse of a week between scrubs appeared to be adequate to restore the flora of the hands to the usual numbers.



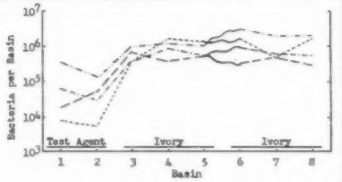
Scrubs on normal hands with no test agent added. —— water only, – – Ivory in second week, ·····Ivory in ninth week, ~~ gloves worn for 2 hours between basins. A, range and average counts of 7 Ivory soap basins without serum in collecting tubes; B, same 7 basins with serum added to collecting tubes.

Fig. 1



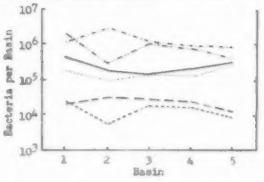
Scrubs on normal hands with test agent in basin 5. ——— Scrubs with pHisohex in basin 5, – – Scrubs with Zephiran, ····· Scrubs with 70% ethyl alcohol.

Fig. 2.



Single surgical scrubs using hexachlorophene test agents – –pHisohex series, ·····Four Star series, – – Germa Medica series, – – Septisol series, ~~ Test agent reapplied and gloves worn two hours.

Fig. 3



Ivory soap scrubs following intermittent daily use of hexachlorophene test agents. —— Green soap series, – – pHisohex series, ····· Four Star series, – – Vestal Antiseptic Liquid Soap series, – – Germa Medica series, – – Septisol series.

Fig. 4

The serum added to some series did not appear to act on the basic counts as an enrichment to increase the counts nor as an inhibitor to decrease them (Fig. 1). The addition of serum to the water from scrubs with the hexachlorophene agents resulted in larger counts than in those without added serum. With the two soaps containing one-fourth per cent hexachlorophene the counts with serum were near the normal range, averaging 350,000, while those without added serum averaged 11,000. However, where the hexachlorophene concentration in the wash water was higher, using the one per cent and three per cent hexachlorophene containing agents, the counts with added serum averaged 20,000, and without serum 5,000. In the scrubs with Ivory soap

following the constant or intermittent use of hexachlorophene the addition of serum to the collection tubes resulted in counts in the normal range, slightly greater in the plates with serum than in those without serum, for the scrubs following the one-fourth per cent hexachlorophene soaps. The counts from the scrubs after use of the one per cent and three per cent hexachlorophene agents were between 6,000 and 100,000 without serum and between 40,000 and 140,000 with serum. The graphic results shown throughout this paper are from counts made without addition of serum.

To check the carry over of various agents from a test basin through succeeding basins of Ivory, counts were made using pHisohex as a scrub and Zephiran or 70 per cent alcohol as soak in the fifth basin (Fig. 2). Though the total count returned to approximately the pre-test levels in the last two basins of each scrub, the proportion of colonies (Table 2) in both the ten and one ml plates after pHisohex remained low, indicating some effective carry over even in six minutes of scrubbing with Ivory soap.

There was an interesting result noted following the alcohol rinse. Two subjects whose counts after an alcohol rinse showed no decrease used an alcohol rinse on their hands or had their hands in alcohol frequently, while three with lowered counts rarely, if ever, had their hands exposed to alcohol. No further qualitative studies of the hands of the two groups were made to discover differences in types of organisms present, or their resistance to alcohol, or differences in the alcohol soluble substances of the skin.

Serial basin scrubs: Single scrub with test agent.—Figure 3 shows the low total counts in the basins of all the test agents. There was great inhibition of growth in the plates containing ten ml of wash water as indicated by the ratio shown in Table 2. After reapplication of test agents and two hours in gloves the counts remained in the normal range of about a million. The standard scrub showed counts even after ten minutes scrubbing that were in the same range as the plain water and Ivory soap scrubs in Figure 1. The series using a Zephiran soak after the test agent gave similar results.

Serial basin scrubs: Cumulative effect.—Figure 4 of the scrubs after intermittent use of hexachlorophene agents shows a range of counts from ten thousand to about a million with two in the ten to thirty thousand range, and one in the range of the standard scrub around two hundred thousand. The counts after constant use of hexachlorophene agents showed the various agents in the same ranges.

The ratios between plates from each basin resembled those of serial basins of Ivory without preparation, in Table 2, except

in the two pHisohex counts and the Four Star count after exclusive use of the test agent, which showed somewhat the same changes as occurred in the basins immedately after pHisohex in Table 2. The ratios for Four Star after intermittent use were of the same pattern in the first two basins, but returned to the Ivory proportion by the third basin.

Discussion

Evaluation of skin disinfectants.—Such *in vitro* tests as the phenol coefficient or other measurement of the rate of killing of bacteria, or physical and chemical characteristics such as ability to lower surface tension or to dissolve fat are inadequate to evaluate skin disinfectants in use. *In vivo* tests for evaluation of the degree of skin disinfection have included methods of swabbing, scraping and excising the skin, contact of the skin with solid or fluid media, culture of washings from the inside of surgical gloves, cultures of test bacteria placed on the skin, hand washings in single or serial basins, and clinical trial. Most methods allow an indeterminate carryover of the test agent into the culture medium. Most do not differentiate between the transient flora which can be removed by mild scrubbing and the resident flora which persist on the skin. Of all these technics the serial basin scrub method of Price (1938) appears to give the most information.

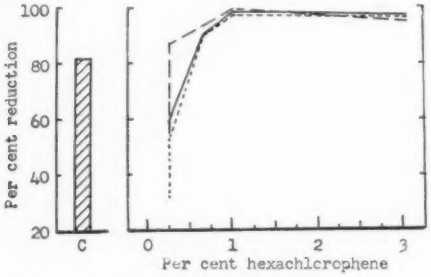
Although the clinical trial might appear to give the final answer, it involves so many variables other than skin disinfection that it is difficult to isolate this one factor. Since most skin carriers of pathogenic staphylococci, for example, are also nasal carriers, any clinical evaluation of a change in scrubbing methods must involve careful control of all masking and capping during both operations and changing of dressings, checks on gloves for punctures, and identification of skin flora of the patients on a series of cases large enough to recognize a change in the proportion of infections.

Alcohol and Zephiran.—Misconceptions are prevalent about the efficacy of alcohol and Zephiran as antibacterial agents. Alcohol in the midbasin of a series of two minute scrubs (Figure 2) not only did not reduce the viable counts significantly, but neither was there any carry over of bacteriostasis even as far as the first scrub after the alcohol soak. In addition, the use of the alcohol as a soak or rinse after a hexachlorophene scrub is contraindicated, since it will remove the alcohol soluble hexachlorophene (Fahlberg *et al.*, 1948) and impair its cumulative effect. Zephiran has inhibitory power when carried over into the culture medium, but does not actually kill the bacteria present on the skin of the hands (Figure 2), and has no cumulative effect (Figure 4).

Effect of hexachlorophene.—In the scrubs where hexachlorophene

was used without any recent previous use, the counts in the test basins with Ivory soap were in the same range as those following standard soap scrubs (Figure 2 and 3). No inhibitory effect of hexachlorophene was evident after a single scrub. The continued use of hexachlorophene in a sufficient concentration showed a marked cumulative effect (Figure 4).

The efficacy of the degerming agents can be expressed as per cent reduction of the counts. The term per cent reduction is probably not an accurate expression of the number of viable organisms remaining on the hands, but is a useful working concept in terms of the number of organisms that can be removed from the skin with comparable treatments. It is merely the proportion of the numbers of bacteria in the test basin to those in the control basin.



Relation of reduction of bacterial count on hands to concentration of hexachlorophene in agents used for preoperative scrub.

C, average of scrubs after standard green soap and Zephiran surgical scrub; -----Scrubs after intermittent use for four days; — — —- Scrubs after constant use for week; ——Average of scrubs after constant and intermittent use.

Fig. 5

The achievement, in less time and with less trauma to the hands, of a percentage reduction comparable to or better than that obtained with the standard scrub can be attained with a liquid soap containing slightly less than one per cent of hexachlorophene (Figure 5). The use of a higher percentage is unnecessary, probably uneconomical, and possibly might be toxic over a long time. The coefficient of correlation between concentration of hexachlorophene and the reduction of bacterial count is 0.5. This indicates that factors other than hexachlorophene are operating. These factors might include the bacteria harboring properties of an individual skin, the species of bacteria in the resident flora, the activities of an individual in the period pre-

ceding the test scrub, or the base in which the hexachlorophene is carried. The amount of soap solids in the use dilution has a subjective effect on the way any soap is used. More than ten per cent of soap is unnecessary and uneconomical, while less than eight per cent does not give good detergent effect in an area with moderately hard water.

A preparation which, in the use dilution, will contain seven-tenths to nine-tenths per cent hexachlorophene and eight to ten per cent soap solids appears to be optimal for use in the surgical scrub from the bacteriological evidence. Consideration of other factors, such as the alkalinity, and the subjective factors of tactility, odor, and color, will be of importance in compounding a product satisfactory to all users.

Summary

1. One possible source of infection of the clean surgical wound is the hands of the operator.

2. A recent addition to the many methods tried for freeing the hands of the operator from bacteria is the use of a soap containing hexachlorophene, the generic name for bis-(2-hydroxy-3,5,6-trichlorophenyl) methane.

3. Tests of the use of hexachlorophene by the serial basin scrub technic showed a decrease in the number of viable bacteria which could be removed from the hands after hexachlorophene had been used for ashing the hands for several days. No greater decrease was found after single scrubs with hexachlorophene containing agents than with soft soap.

4. The optimum per cent of hexachlorophene in the use dilution of the soap for daily washing was found to be 0.7 to 0.9 per cent. This gave a reduction in the bacterial count of over ninety per cent for a four minute scrub, compared to reduction of eighty-two per cent for the standard ten minute scrub with Zephiran soak.

Acknowledgment

The authors appreciate the assistance of the volunteers who participated in this study, particularly Miss Dorothy Schmidt, R. N., Miss Hazel Nash, R. N., and Otto Tripple, M. D.

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ABSTRACT

OBJECTIVE EVALUATION OF PATIENTS WITH RHEUMATIC DISEASES I. COMPARISON OF SERUM GLYCOPROTEIN, COLD HEMAGGLUTINATION, C-REACTIVE-PROTEIN AND OTHER TESTS WITH CLINICAL EVALUATION

M. R. Shettar et. al. (Veterans Administration Hospital, Oklahoma School of Medicine) J. Lab. & Clin. Med., 48, 194-9 (1956).

Patients with rheumatoid arthritis were classified according to the criteria of Steinbrocker and associates, in addition to which a rating of the current clinical activity was recorded at each visit of the patient. Normal controls were comprised of hospital personnel. Serum glycoprotein, C-reactive protein, cold hemagglutination, antistreptolysis O, erythrocyte sedimentation rate, and gamma globulin levels were compared in this study.

The hemagglutination test shows significant correlation with the acute phase reactant tests including glycoprotein, C-reactive protein, and sedimentation rate, or with clinical activity of the rheumatic process.

The serum glycoprotein concentration and certain other acute phase reactant tests which are less specific for the rheumatic diseases serve as indication of clinical activity. C-reactive protein concentrations, although not as well correlated with clinical activity as glycoprotein, are more easily determined and thus may be more readily adapted to the routine laboratory.

Serum glycoprotein and C-reactive protein were significantly correlated with clinical activity. A correlation barely significant at the 5% level was found between sedimentation activity. Neither glycoprotein nor C-reactive protein was significantly correlated with sedimentation rate. Serum gamma globulin was generally elevated in patients with rheumatoid arthritis but was not correlated with rheumatoid arthritis.

ANTIBODY RESPONSE TO PROTEUS MIRABILIS ISOLATED FROM INDIVIDUALS WITH AND WITHOUT THIS INFECTION*

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With the widespread use of antibiotic therapy, secondary infections with organisms resistant to these antibiotic drugs are becoming more apparent. One of the most common organisms isolated from the stool, urine, and other specimens following prolonged antibiotic therapy is Proteus mirabilis.

The suppression of the other bacteria by widespread use of antimicrobial agents has increased the relative importance of Proteus and Pseudomonas as causative agents in infectious processes. Cases of infections due to these organisms that developed during or following repeated courses of antibiotic therapy are reported. The relationship of the infection to the antibiotic therapy was believed in most patients to be a matter of change in bacterial flora owing to the elimination of sensitive bacteria and multiplication of resistant strains. The possible seriousness of giving the broad spectrum antibiotics prophylactically must be considered, since if an infection does occur with a nonsensitive organism, it is almost certain to constitute a difficult therapeutic problem. Streptomycin in combination with large doses of sulfonamide was the most effective therapy in Proteus infections. The discontinuance of ineffective antibiotics was sufficient in some patients to control the infection by allowing the re-establishment of normal bacteria flora.[4]

The Proteus species generally have been recognized as harmless organisms, but there is increasing evidence that organisms in this genus may produce severe illness, either primary or secondary. Weiser[3] conducted a bacteriologic study of the stools of forty infants under one year of age who had diarrhea and found Proteus mirabilis consistently. Each patient was examined several times during the illness. Proteus mirabilis also was isolated from the blood stream of a seventy-year-old woman who had subacute bacterial endocarditis; and, since no other organism was isolated, this was assumed to be the cause of death.

Strains of Proteus mirabilis that were apparently identical biochemically and serologically were isolated from suspected ham and from nine of nineteen patients who became violently ill with gastro-enteritis after eating the food. Attempts to demonstrate a causal relationship of Staphylococci or Streptococci were unsuccessful. It is believed that the studies furnish strong cir-

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

cumstantial evidence of the probable etiological relationship of Proteus mirabilis.[5]

The finding of gram negative rod bacteria such as the coliforms, paracolons, and Proteus species, etc., raises the question of etiologic significance since these organisms may be isolated from the stool, skin, and other areas in healthy individuals; and, of course, they may occasionally become invasive. A number of workers have pointed out that tissue invasion by these gram negative rods stimulates antibody formation, and this may be used as a clue of significance.[6, 7, 8] The work in this regard has been done mainly on the paracolon groups. Brown,[6] reporting from Adelaide, Australia, points out that finding specific agglutinins in the patient's serum against the organism isolated from the stool is convincing evidence of pathogenicity. She isolated anaerogenic paracolon bacilli from the feces of thirteen children ill with gastro-enteritis. The etiology was supported by the presence of specific agglutinins for the group in the sera of the patients. Serial agglutination tests on the serum from one child showed a rise and fall of titer as the severity of the infection receded.

The purpose of this paper is to determine if agglutinin antibodies against Proteus mirabilis are increased in the patient's serum following isolation of this organism and to learn if these antibodies may be demonstrated in the serum of so-called normal individuals, that is from blood donors. These studies were performed under controlled conditions.

Methods

The method employed to detect the antibody response, if present, was set up to conform with the following protocol as described by Zinsser and Bayne-Jones.[1]

Tube	Antiproteus Serum Dilution (Total Amt. 1cc.)	Suspension of P. Mirabilis c.c.	0.85 Percent NaCl	Final Serum Dilution	Incubate 56° 2 Hrs.
1	1–10	0.5	..	1–20	Refrigerate
2	1–20	0.5	..	1–40	overnight
3	1–40	0.5	..	1–80	"
4	1–80	0.5	..	1–160	"
5	1–160	0.5	..	1–320	"
6	1–320	0.5	..	1–640	"
7	1–640	0.5	..	1–1280	"
8	1–1280	0.5	..	1–2560	"
9	1–2560	0.5	..	1–5120	"
10	(Control)	0.5	0.5	....	"

The antigen was a twenty-four hour culture of Proteus mirabilis in a meat extract broth, the Proteus having been isolated from the patient. Emulsions of the bacteria were made by taking up growth from triple sugar iron agar slants and transferring them to an 0.5% solution of formalin. This solution was then

placed in a 56° C water bath to kill the organisms. The emulsion should have a density of about 0.5 on the McFarland nepholometer scale and contain approximately 500 million bacteria per c.c. The time in the 56° C water bath was two hours.

The McFarland nepholometer was prepared by making a 1% aqueous solution of C.P. sulphuric acid and a 1% aqueous solution of C.P. barium chloride. Then to a series of ten test tubes of uniform size, increasing amounts of barium chloride solution was added starting with 0.1 cc in the first tube, increasing the amount by 0.1 cc in each succeeding tube so that 1.0 cc is added to the tenth tube. Enough sulphuric acid solution was then added to each tube to bring the total volume to 10 cc. The tubes were sealed and labelled serially 1 to 10.

If broth cultures are used, the 10% barium chloride is prepared with broth instead of water.

The density of the suspensions in these tubes corresponds approximately from 300 million organisms per cc for the first tube to 3000 million organisms for the tenth tube, increasing by 300 million bacteria for each succeeding tube 1 to 10.[2]

TABLE I

Serum Agglutination Patterns Following the Isolation of Proteus Mirabilis

Patient	Diagnosis	Nature of Specimen Cultured	Serum Titer Against Isolate
A. B.	Congestive failure	Stool	Neg 1:20
C. D. Y.	Fractured hip	Stool	Neg 1:20
J. McD.	C. V. A.	Urine	Neg 1:20
C. C.	Benign Pres. Hyper.	Urine	**Pos 1:2560**
F. F.	Pneumonia	Stool	Neg 1:20
I. C.	Gastric enteritis	Nose & Throat	Neg 1:20
L. McN.	Meningitis	Stool	Neg 1:20
E. C.		Wound	Neg 1:20
D. R.	Appendicitis	Abdominal fluid	Neg 1:20
A. S.	Congestive failure	Stool	**Pos 1:40**
E. S.		Urine	Neg 1:20
M. R.	Luetic heart? C. V. A.	Vaginal discharge	**Pos 1:40**
L. G.	Mastoiditis	Nose & Throat	**Pos 1:80**
J. C.	Transverse myelitis	Stool	Neg 1:20
C. F.	Hernia	Urine	Neg 1:20
D. D'E.	Gastric enteritis	Stool	Neg 1:20

Results

The agglutination studies reported in Table I were performed on sixteen patient's sera. Seven of the specimens were obtained from stools, four from urine, two from the nose and throat, one from a wound, one from abdominal fluid, and one from a vaginal discharge. Twelve of these specimens did not demonstrate antibodies against Proteus mirabilis in a dilution of 1:20. Four were positive for these antibodies, one in a 1:2560 dilution, two in a 1:40 dilution, and one in a 1:80 dilution.

In Table II is listed the control specimens obtained from blood donors' sera and tested with the antigens of the patients listed

TABLE II
Detection of Agglutinins Against Proteus Mirabilis in Serums of Normal Individuals

Patient	Titer
1	Neg 1:20
2	Neg 1:20
3	Neg 1:20
4	Neg 1:20
5	Neg 1:20
6	**Pos 1:80**
7	Neg 1:20
8	Neg 1:20
9	Neg 1:20
10	Neg 1:20
11	Neg 1:20
12	Neg 1:20

in Table I. All were negative 1:20 except one which was 1:80 positive.

Comment

In the series of sixteen patients from whom Proteus mirabilis was isolated, increased serum agglutinins against the particular isolate was demonstrated in four instances. One individual had a serum titer of 1:2560; this undoubtedly indicates antibody stimulation evoked by tissue invasion. Several reasons may be offered for the failure of the other twelve patients to show antibody against their Proteus mirabilis isolate. The organism may have been a secondary commensal without actually causing tissue injury or invasion, or the serum may have been collected before sufficient time had elapsed to allow antibody stimulation, or the antigen may have been refractory to agglutination because of interferring surface components.

From the studies made of Proteus mirabilis, it appears that this organism is capable of invading tissue and producing infections. However, it would not be sufficient to assume that its presence was evidence of infection, because several patients with a low titer or no titer at all showed no clinical signs of bacterial invasion.

Although this paper is not complete, it seems the clinical manifestations must be considered before any significance is attached to the presence of Proteus mirabilis. If this organism is the predominating one and the patient does have the clinical signs of an active infection, then it would be valid to consider Proteus mirabilis to be the etiological agent.

Summary

Agglutination tests were performed on the serum of sixteen patients from whom Proteus mirabilis had been isolated in an attempt to determine etiologic significance of this organism. Four of the sixteen patients showed no increase in agglutinin titer, one being positive at 1:2560. Similar tests done on the serum of twelve normal individuals showed no detectable agglutinins except in one instance where the titer was 1:80.

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ABSTRACTS

HEMOGLOBIN J

Oscar A. Tharp et al. Science 123, 889-90 (1956)

A third hemoglobin which has a higher electrophoretic mobility than normal adult hemoglobin at pH 8.6 but differing from hemoglobins H and I, adds a tenth abnormal human hemoglobin. The father and six siblings showed this same abnormality, the sickling phenomena and fetal hemoglobin were absent. There was no evidence of hematologic difficulties and physical examination showed no abnormalities attributable to this abnormal component.

SERUM LIPASE DETERMINATION: FOUR HOUR TECHNIC WITH OLIVE OIL SUBSTRATE. Leitha D. Bunch and Richard L. Emerson. (H. L. Snyder Memorial Research Foundation, Winfield, Kans.) Clin. Chem. 2, 75-82 (1956).

A method for determining serum lipase activity requiring only 4 hours incubation at 37° C is given. The details of the 4 hour technic are given along with results in serum from healthy controls, patients with pancreatitis, and patients with other pathologic conditions.

Reagents used are: Olive oil emulsion, M/15 phosphate buffer, 9:1 alcohol-ether, N/10 NaOH. Just before starting the lipase determination, mix 5 volumes of the M/15 phosphate buffer and 1 volume of olive oil emulsion by stirring. The final pH of the buffered substrate is adjusted to 7.5 with HCl or NaOH. Measure 12 ml. portions of the prepared substrate into test tubes, using 2 tubes for each determination. Warm the tubes of the substrate to 37°C in a water bath. Add 1 ml. of serum to one of the tubes and mix the serum and the substrate thoroughly by inversion. The second tube serves as a blank. Both tubes are incubated at 37° C for 4 hours. Immediately after incubation, add 1 ml. of the serum to the blank. Both the test and blank mixtures are inactivated by the addition of 50 ml. of 9:1 alcohol-ether. The inactivated mixtures are titrated with N/10 NaOH to a pH of 10.65, using the Beckman Model G pH meter to determine the end point. The volume of NaOH used to titrate the blank is substracted from the test titration figure, the final result being expressed as the volume of N/20 NaOH needed to titrate the fatty acid liberated in 4 hours by 1 ml. of serum. The values found in 57 healthy controls ranged between 0.06 and 0.87 units, with a mean of 0.31 plus-minus 0.17 (S.D.)

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: Case Report with Comments upon the Urinary Iron Loss. Samuel I. Rapport, M.D., Emmett B. Reilly, M.D., Norman R. Eade, M.D., and Herbert O. Carne, M.S. (Veterans Administration Hospital, Long Beach, California) Ann. Int. Med. 44, 812-18 (1956). Paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli Syndrome) is a rare chronic hemolytic anemia characterized by intravascular hemolysis and the urinary excretion of hemoglobin and hemosiderin. An acquired defect of the red blood cell stoma causes the patient's erythrocytes to be destroyed by a normal plasma factor or factors, possibly the newly described euglobulin, properdin.

A white male patient with parozysmal nocturnal hemoglobinuria is described who had low serum iron levels and hypochromic red blood cells, probably due to the steady loss of iron in the urine over a period of 17 years. His urinary iron output was measured over a three month period. At times it exceeded 20 mgms. per day. The average loss was 4 mgms. per day. The manner in which iron is lost in the urine in this disease is discussed.

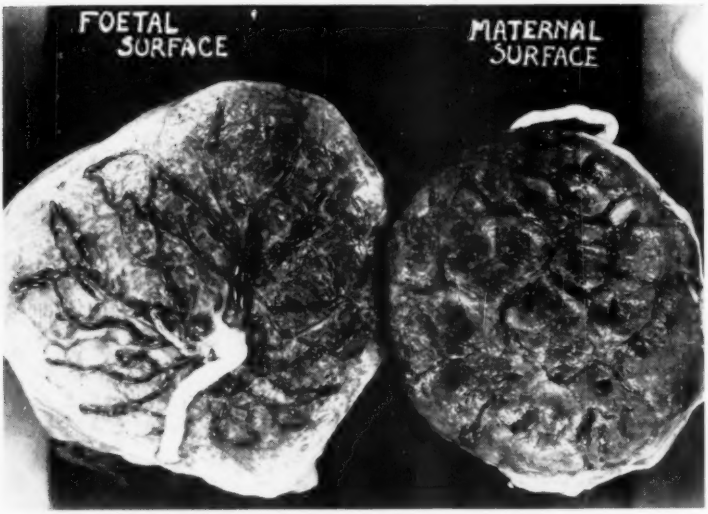
A NEW TECHNIQUE FOR THE DEMONSTRATION OF THE FOETAL CIRCULATION*

MR. ANDREW FRASER, F.I.M.L.T.

Introduction

The human placenta is neither an easy structure to understand nor to investigate. It is rather ironic that so little is known about this organ on which the continuity of mankind is utterly dependent. As late as 1950 it was stated by Hamilton and Boyd that our knowledge of the intimate structure and growth of the placenta is still only a first approximation.

The external appearance of the placenta is familiar to all who practice obstetrics and has been described by many authors (Plate I). The internal anatomy is however still a matter for dis-



Demonstrates the foetal and maternal surfaces of a normal fresh, untreated placenta with the foetal membranes removed.

cussion and there is a wide divergence of opinion. It may be asked why so little is known about the anatomy of the placenta when other organs of the body, and seemingly more complicated, have had their structure demonstrated. The explanation of this anomaly lies in the innate structure of the placenta. It is not easy to dissect. The foetal vessels present, as the dissection is continued, an ever increasing network of divisions and ultimately dissection must

* Chief Technician, Research Department, Royal Maternity and Women's Hospital, Glasgow, Scotland. Read before the First North American Conference of M.L.T., Quebec, June, 1956.

be abandoned or becomes so inaccurate that it is of little value. Dissection has, of course, been attempted and Bumm in 1893 published an account of placental anatomy based on many years of patient dissection. He visualized the placenta being composed of tree-like fronds or villi extending from the foetal chroion to the maternal decidua. Some of these fronds were attached to the decidua but the majority floated free in the maternal blood. This was a simple arrangement, easy to understand, and it remained an accepted fact until Spanner in 1935 proposed a completely new idea of placental anatomy. After Bumm the method of injecting the placental vessels with dyes or 'fillers' such as gelatine or celloidin came to be used and various papers were published on the findings obtained by such methods. These results were a great advance in so far as they showed the mode of division of the placental arteries and veins, but finer details could not be defined. A further advance was made by the use of fillers followed by corrosion of the placenta with acid leaving a cast of the vascular tree. This injection-corrosion technique was a great advance. Not only could the mode of division of the placental vessels be seen, but the cast was in three dimensions and the relationship of the vessels to each other could be appreciated. The foetal veins were observed to accompany the arteries more or less regularly. The objections to the injection-corrosion technique can be stated at this point and they are quite formidable. In the first place the placental vessels must be washed out with normal saline solution to get rid of foetal blood. This can produce vessel rupture and subsequent leakage. Thereafter, as a preliminary to the actual injection of the chosen 'filler' the foetal vessels must be washed out once again with acetone to prevent premature hardening of the filler and ensure its farthest possible penetration. Acetone denatures vessel walls and makes them still more liable to rupture. The fillers usually employed have a certain viscosity and considerable pressure is necessary to force the filler along the vessel lumen. As a result of this and the preliminary treatment the filler usually leaks and the cast is often distorted. Artefact is an ever present possibility and since the foetal vessels are removed by the subsequent corrosion there is no way of checking the accuracy of the cast.

In 1935 Spanner after ten years of investigation, both of delivered placentae and placentae in situ, published an account of the foetal placental circulation. His views have been widely adopted and are still the basis of standard teaching in most countries. He used mostly liquid latex as the filler and studied not only corrosion casts but also injected placentae in situ. His results were excellent and no one has obtained better preparations with this technique Similar studies were made by Romney and Reid in 1951 and by Wilkin in 1954, but in all of these studies the same objections to

the injection-corrosion technique will hold good. The danger of artefact is ever present.

The need for an accurate picture of placental anatomy is not just an academic quest. An understanding of placental anatomy would permit in turn, an understanding of the pathological lesions observed in the placenta, an understanding of clinical results particularly in relation to foetal death or survival and last but by no means least, an understanding of placental growth.

The method now to be described is free from most of the objections which beset the injection-corrosion techniques and allows a more complete demonstration of placental anatomy than has been possible so far.

In histo-chemistry it is the practice to use trypsin for the identification of protein. Also, in pathology it is found that tissues vary in their resistance to the digestive activity of trypsin, connective tissues being usually more resistant than parenchymatous. In this fashion the idea was conceived of digesting the placenta and removing parenchymatous tissue whilst preserving connective tissue such as blood vessels. Since proteolytic enzymes are the most important, trypsin was finally selected.

Preliminary experiments showed that it was possible to remove decidua, chorionic epithelium and mesoderm leaving behind the foetal vessels, chorion and umbilical cord. Subsequent work, however, has shown that the technique must be modified according to the type of vessel it is desired to study, and the duration of digestion watched carefully. If the main structure of the vascular tree is to be studied, digestion may be allowed to proceed unchecked, but removal of all support from the vessels in this manner renders them liable to damage and it is difficult to demonstrate the finer ramifications of the vessels. This is particularly so in relation to the terminal capillaries. These are extremely fragile and if not already attacked by the enzyme may easily be swept away in any subsequent washing or handling unless extreme care is exercised. It has even been found possible to limit the digestion to the decidual layer leaving the villi complete with their covering of chorionic epithelium. This has greatly facilitated the study of the villus and its constituent capillary. Following digestion with trypsin the placental vessels may be injected with a coloured gelatin solution and subsequently hardened and preserved in formalin.

When digestion and injection are completed the placenta is a permanent preparation and can be handled and dissected without difficulty.

Materials

Placentae. Only fresh placentae were used and were digested usually within a very few hours of delivery. If any delay was anticipated the placenta was refrigerated until required. The object of

using fresh material was to guard against any possibility of artefact by decomposition. Round glass jars sufficiently big to accommodate the whole placenta and allow it to lie flat without distortion were employed. For digesting single lobes or smaller pieces smaller vessels were used; the important point being to adjust the size of the vessel to the size of specimen and so keep all the ensymes in contact with the tissue.

For injection of the specimens following digestion, polythene catheters were employed, of a diameter small enough to enter the umbilical vein and arteries. It is of advantage to cut the catheters straight across and not to a point. The vessel walls are thin and can be penetrated easily.

A large hypodermic syringe, of at least 50 ml. capacity, was used for the actual injection.

A one per cent solution of a water soluble opaque dye made by Imperial Chemical Industries was found to be most satisfactory, "Monolite" for green, yellow and red; "Monastral" for blue. The arteries and veins may be injected with red and blue dyes respectively. These dyes can be used alone or dissolved in fifteen per cent gelatine solution.

The digestion solution. This consists of a one per cent solution of sodium carbonate, and is used to immerce the whole placenta or a lobe as required. Then to the alkaline solution is added sufficient trypsin powder (B.D.H.) or liquor trypsin compound, (Allen and Hanbury), to produce a one per cent solution of digest.

The Method of Digestion

(a) *The Placenta.*

A fresh placenta is washed carefully in cold running water to remove blood clot, and the foetal membranes are pared off around the placental edge. The cord is cut short but should not be less than three to four inches in length. The placenta is placed in a glass container, maternal surface downwards and covered completely with the solution of digest. The container is covered with a lid and the placenta incubated at 37° C for periods varying according to the structure to be examined. It is of advantage to inspect the rate of digestion frequently during the digestion period. It has been found that the activity of commercial trypsin varies and digestion may proceed more rapidly than anticipated with a loss of specimens. A fume chamber, in which the incubator can be placed, is of immense advantage, because at the end of digestion the odour is offensive.

When digestion is complete the placenta in its container is placed in a larger vessel into which a gentle stream of cold water is led. It is important that the water should not run directly onto the placenta. After a few hours the digested material will have been washed away. If it is considered that further digestion is necessary the placenta can easily be replaced in the incubator

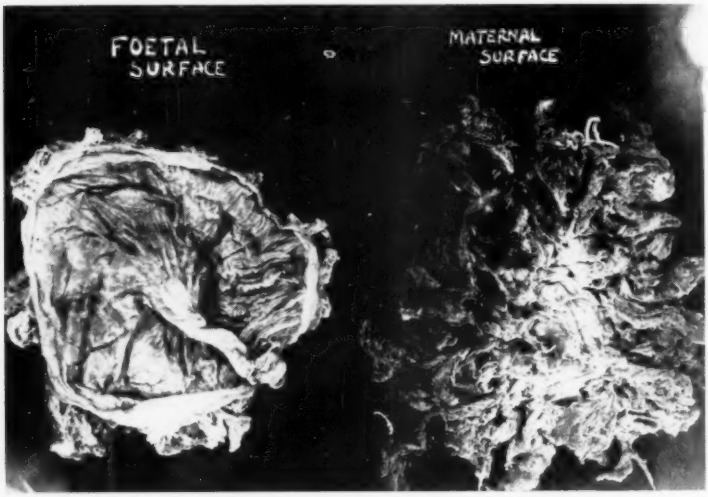
with fresh digest solution and the digestion continued. When digestion is complete the placenta consists only of umbilical cord, chorion, and cotyledonary vessels.

The specimen is next immersed in 10 per cent formol saline for approximately 1 hour. This hardens the tissue somewhat without producing distortion. Thereafter the specimen is immersed in warm water. The umbilical vein and arteries are catheterised gently and gelatin solution, suitably coloured blue or red, injected into the vein and arteries. The gelatine should be at a temperature of fifty degrees centigrade or more to ensure the fullest penetration of the solution. An ordinary 50 ml. hypodermic syringe is quite suitable and gentle finger pressure sufficient. Excessive pressure should be avoided. About 100 ml. are required for each artery and vein, and the quantity should be too little rather than too much. Injection should be carried out rapidly and when completed the placenta is placed in a cold solution of 10 per cent formol saline for at least another hour before being handled. (Plates 2 and 3.)

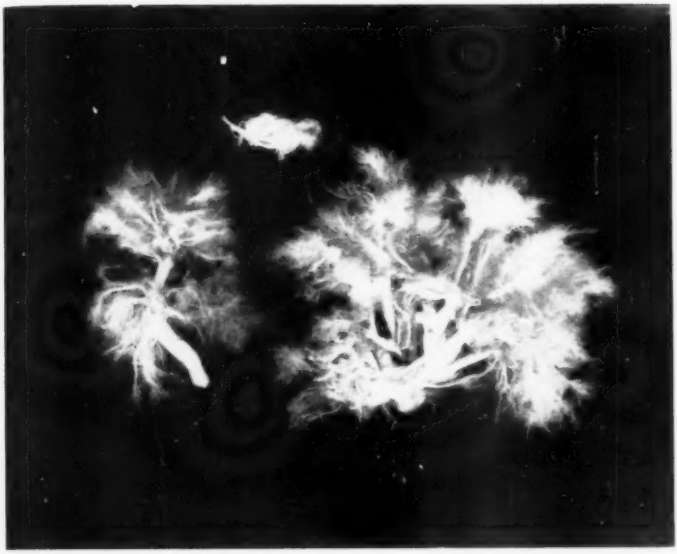
(b) *The capillary vessels.*

A rather different technique is necessary to demonstrate the capillaries of the villi.

The placenta is washed as before in cold water and a suitable lobe selected. The main artery and vein supplying this lobe are



Demonstrates the foetal and maternal surfaces of a digested and injected placenta. Note that on the maternal surface only the injected cotyledonary vessels remain. These specimens can be handled and manipulated with ease and without risk of damage.



A photograph (life size) of 3 cotyledons of varying sizes dissected from a digested placenta.

This illustrates the ease with which even the finest vessels can be demonstrated and any portion of the vascular tree can be studied in detail by simple dissection.

injected with a one per cent solution of red and blue dye respectively with the addition of a small amount of one per cent solution of sodium carbonate. The alkali appears to favour penetration of the dye. A fine hypodermic needle is used and the vessels entered directly in the usual way as for a venepuncture. About 20 ml. are sufficient in each case.

The lobe is now cut out of the placenta and placed in a small glass jar or container with the maternal surface uppermost. This is most important. It allows the vessels to float freely in the digest solution and permits examination without disturbing the specimen unduly. Digest solution is added and the lobe incubated for not more than twenty four hours at 37° C.

At the end of this time the container is opened and with great care the digest solution is siphoned from it and replaced with ten per cent formol-saline. Formol-saline hardens the capillary vessels and makes them more amenable to dissection. The specimen can be examined at once but storage in a refrigerator for considerable periods does no harm provided the lobe is kept moist with formol-saline.

By sharp dissection very small pieces of tissue are cut from the

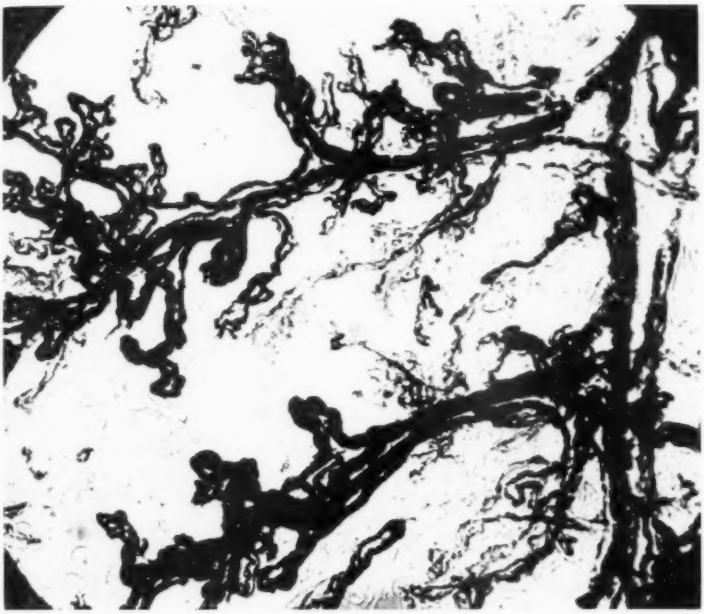
surface of the lobe and placed on a glass slide which has been moistened with water. After digestion pieces may be dissected from a cotyledon at any level from the chorion outwards, thus allowing a complete picture of capillary distribution to be built up. Before proceeding with the mounting of the specimen, it should be examined with a low power objective to ensure that the area is well coloured, both red and blue. Glycerine jelly is dropped on the specimen and a glass cover slip placed over it. Gentle pressure with the point of a fine hypodermic needle is now exerted over the area of tissue under the cover slip. The material will be seen to flatten and gradually spread out. Pressure is continued until it is apparent that no further spreading will take place and also until the jelly is sufficiently hardened to keep the material in its present position. The preparation is now ready for examination. This pressure method of mounting has been found to be much easier and more satisfactory than dissection under direct vision.

The placental capillaries are, in life, closely enmeshed with each other and the difficulty in their examination has been to devise a simple method of separating them without causing damage. Pressure does this in a satisfactory fashion and allows individual capillary vessels to be seen in their entire course. The digestion can be controlled in such a manner that only the maternal decidua and fibrinoid layer is affected thus permitting the retention of the syncytial membrane around the capillary. This gives a satisfying picture of the capillary vessel. (Plate 4.)

Discussion

In the practice of histology, as already related, trypsin and other proteolytic ferments have been employed to identify protein in histological sections. The application of this principle to produce a technique which would demonstrate the foetal placental circulation, is quite new and a marked departure from previous techniques. The placental preparation which is obtained after digestion is extremely suitable for demonstrating the anatomy of the placenta. The mode of technique has been described in detail.

The placental preparation has several advantages over the cast of the foetal vessels which is obtained by the usual injection-corrosion technique. The principle advantage of this method lies in the fact that the foetal vessels are preserved and can be used to check the results. No such check is possible when the foetal vessels have all been corroded and removed by acid. It follows that artefact can be guarded against and eliminated. There are other advantages. For example the method is much quicker and easier to perform. A placenta can be prepared completely within three days as against the seven or more days required by the injection-corrosion technique. In addition few placentae need be rejected because of vessel rupture and spilling of filler. The



This is a microphotograph of the capillary loops, mag. x 350. The red dye was injected into the umbilical artery and the blue dye into the umbilical vein.

method also is quite certain and consistent in its results provided the basic technique is applied correctly.

From the view point of scientific investigation the digested placenta is most suitable. The actual vessels can be seen and dissection carried out with accuracy. Cotyledons can be separated from their neighbours under direct vision and individual cotyledons dissected into their constituent sub-cotyledons. This accurate dissection permits the investigator to obtain a clear picture of the relationship of cotyledons to each other. This is most important in a three-dimensional structure such as the placenta.

In conclusion my thanks are due to Dr. A. D. T. Govan, Director of Research, Royal Maternity and Women's Hospital, Glasgow; to Dr. J. M. Crawford, Obstetrician and Gynaecologist, County of Lanark, and to Dr. J. Hewitt, Obstetrician and Gynaecologist, Royal Maternity and Women's Hospital, Glasgow, for their great assistance and encouragement in the preparation of this paper.

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NEW FRONTIERS*

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In our ever changing world of this twentieth century, we hear much concerning "new frontiers." We read of the "new frontiers" of this atomic age and man's attempt to harness the power of the atom for practical use. Then, too, we find the development of man-made missiles which travel faster than the speed of sound into frontiers never before reached by man. In a sense, medicine has its "new frontiers" as well—for the conquest of disease and its effects is a never ending task for the pioneers, like Salk of today—and Pasteur, Lister and Koch—pioneers of the medical frontiers of the past. Similarly, the profession of medical technology is not static, for new methods, techniques, devices and even new fields are constantly being developed.

Surgery of the heart is one of those new medical "frontiers" I speak of—and, from my ringside seat directly behind the surgeon's shoulder, I was fortunate to be able to observe several of these miraculous feats.

Of especial interest in these "new frontiers" for medical technologists are the cardiopulmonary laboratories now being established in many of our larger hospitals. The main objectives of these special laboratories are to solve the diagnostic problems of cardiac diseases and defects, as well as to perform surgery to correct these defects in cases where surgery is possible.

I was fortunate to spend part of my rotation elective period, of my senior year in medical technology training, in the Cardiopulmonary Laboratory at Children's Hospital in Denver. This laboratory is staffed by an M.D., two resident assistants, and a registered medical technologist. By obtaining electrocardiograms on defective-heart patients, along with heart catheterizations and special blood studies, this team can 'usually' determine the type of defect or defects of the patient, as well as their location.

Some authorities have stated that a well trained cardiologist can diagnose congenital heart disease accurately in 95% of all cases without need for catheterization with just the medical history of the patient, a physical examination, an electrocardiogram, and fluoroscopy. However, this does not mean that cardiac catheterization is not employed in any of those 95% of all congenital heart disease cases, for catheterization does provide a useful tool in establishing the degree of the severity of the defect.

Following a physical examination of the patient, electrocardiograms (tracings of the electrical impulses of the heart) are taken by the medical technologist and interpreted by a specially trained

* Third Award, Scientific Products Foundation—Professional 1956. Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

physician. General knowledge as to the type and magnitude of the defects can be estimated from these tracings. Next, if it is deemed necessary, heart catheterizations are performed to determine the location and extent of the defects.

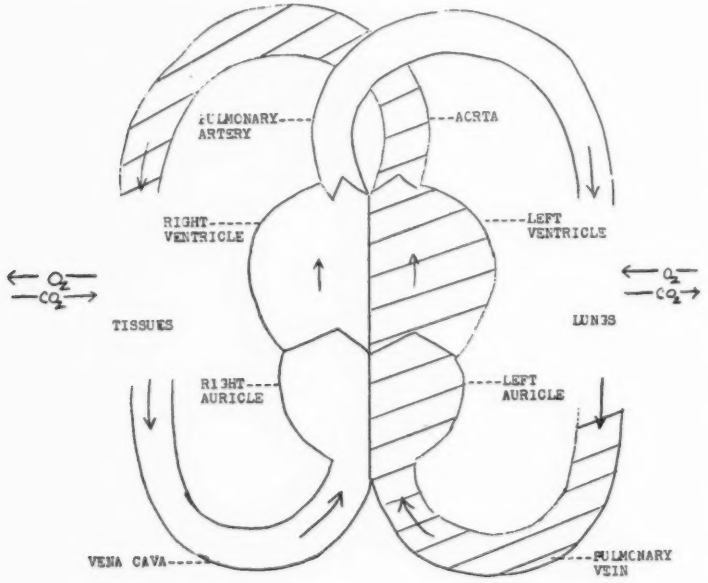
In this surgically sterile procedure, a small plastic catheter is placed in an arm vein and guided by a fluoroscope through the vein into the different chambers of the heart. Pressure tracings of the various areas of this organ are recorded on an oscilloscope and later analyzed. From these tracings, this team can determine which areas of the heart are overworking to compensate for the defect and also obtain other information as to the workings of the heart. In addition to these pressure tracings, measurements of the oxygen content of blood obtained from these various areas of the heart, are also carried out. In the latter procedure the heart blood passes through a cuvette and its oxygen content is registered on an oxymeter.[1] There are no established normal values for oxygen content of the blood, for each individual has a different oxygen capacity depending on his or her hemoglobin content. However, if the oxygen containing capacity of a particular blood sample is determined, along with the actual oxygen content of that sample, the per cent saturation of the blood in that area of the heart can be determined.

The medical technologist obtains blood samples from the various areas of the heart and, using a modified micro Scholander technique, determines the oxygen content of each sample. This specific determination makes use of a micro-syringe and consists of the release of the oxygen from a measured amount of blood by the addition of urea.

In carrying out this procedure, the blood samples are taken under sterile mineral oil to avoid contamination with room air, and a measured amount of a particular sample is treated with sodium hydroxide. The sodium hydroxide removes the carbon dioxide present, and the volume of the remaining gases can be easily read. Pyrogallol is next added to remove the oxygen, and a second reading is taken, which is compared with the first. A blank is also run to determine the oxygen content of the reagents, the results being used in the final determination.

To determine the oxygen capacity of the sample, tank oxygen is passed through the sample of blood to completely saturate it and the above micro Scholander technique repeated, thus obtaining the oxygen capacity value. To determine the per cent saturation of the sample, the oxygen content is divided by the oxygen saturation and the quotient is multiplied by 100.[3] Normally, arterial blood has a saturation of 95% at sea level, with venous blood being about 70% saturated.[4] At the altitude of Denver the per cent saturation can be lower, 90% being the borderline between normal and abnormal.

The values for per cent oxygen content are of great diagnostic importance. For instance, if a patient had an atrial septal defect (an abnormal opening in the septal wall between the two auricles) the blood in the left atrium would be normally saturated, while the blood in the right atrium would have a higher oxygen content due to a flow of blood from the left atrium into the right atrium.[1] From the combined data of pressure tracings, blood oxygen content and capacity, and per cent oxygen saturation, the location and extent of defects is unearthed and the advisability of surgery determined.



To clarify the actual physiology of the heart, and the passage of the blood in its never ending journey through the body, a schematic drawing of this organ is included in this report. Blood from the tissues, laden with carbon dioxide and wastes, enters the right auricle via the superior and inferior vena cavae and passes on into the right ventricle and into the lungs via the branched pulmonary artery. In the lungs the wastes are replaced by oxygen, and the journey continues to the left auricle via the pulmonary vein, on into the left ventricle, and out into the tissues via the aorta, the largest artery of the body.[4] Without valves, capillaries, diaphragm and various other muscles, the heart could

obviously never function properly. In the normal individual then, the blood is high in oxygen content when it is in the left side of the heart and low in oxygen when in the right side.

There are any number of conditions, either congenital or acquired defects—which may bring about a need for heart surgery. The more important congenital defects usually repaired or attempted by the particular surgeon whom I observed are described below.

A pulmonary stenosis is a constriction or blockage of the pulmonary artery. "Patent ductus" is a term denoting a joining or connection of the aorta and the pulmonary artery. An interventricular septal defect is an opening in the walls or septa between the two ventricles, while an interatrial septal defect is the same condition in the septum between the two auricles, the former being the more difficult to repair. A tetrology of Fallot consists of four heart defects: 1) an interventricular septal defect, 2) the aorta overrides the ventricular septum, 3) pulmonary stenosis, and 4) hypertrophy of the right ventricle.[4]

Following is a brief explanation of the surgical procedure currently used at this particular hospital. By placing the anaesthetized body in a "bassinet" type container of ice cubes, the patient's body temperature is lowered to reduce the rate of heart beat to about half the normal time, simultaneously diminishing the general metabolic rate. The body, except for the head, is covered with ice. As soon as the body temperature, which is registered on an esophageal thermometer, reaches 85°F. (usually 1-2 hours) the body is returned to the operating table. A transverse incision is made across the entire chest area at the fourth interspace and all blood vessels tied. The heart is then exposed, and, just before the heart musculature is cut, the channels to and from the heart are occluded. The superior and inferior vena cavae are closed first—then, after a few beats, the loops of tape are tightened around both pulmonary roots, and finally a clamp through the transverse sinus of the heart is closed to shut off the aorta and pulmonary artery.[5] The patient can remain in such a condition only a short time, as brain degeneration may result if the brain is deprived of oxygen for too long a period. Consequently, the surgeon must be extremely dextrous and work rapidly in sewing the defects, or opening them, as the case may be. The heart is seldom open for longer than 4-8 minutes for this particular type of heart surgery.[5] When repairing an interventricular septal defect, another doctor pumps freshly oxygenated blood directly into the heart, while the actual heart surgery is progressing.

The defects having been repaired, the heart and pericardium are sewed up, the rib cage is closed and the chest incision is sewed up. The patient is then placed in a large 115°F. incubator

and his temperature is gradually brought back to normal. This entire procedure, from the beginning of the administration of anaesthesia to the semiconscious state after the temperature is brought back to normal, takes approximately eight to ten hours. If the surgery is successful, the patient is up, and ready to begin his new life in about ten days—his "new life" with a "new heart."

The oxygenated blood I mentioned above is usually used only in cases of interventricular septal defects and is obtained by the following procedure. Two donors whose bloods are compatible with the blood of the patient are readied for blood collection by immersing the arm from which the blood is to be drawn in a 115°F. water bath. This high temperature speeds the rate of circulation so that little oxygen is lost from the blood into the tissues. Consequently, when the blood is drawn from the donor, after this fifteen minute soaking period, it contains a much higher concentration of oxygen than blood collected under normal conditions.

This surgical technique for correction of heart defects has proved quite successful at this particular hospital, yet many problems still exist. To solve these problems, especially the problem of hemorrhage after the surgery, special blood studies are being made by the medical technologist in this particular cardiopulmonary laboratory. In these studies, four blood samples are drawn; one after the patient has been anaesthetized (used as patient's normal); another at the lowest temperature before the chest is opened; a third after the chest has been closed and the temperature is beginning to rise; and finally another when the temperature is back to normal. Prothrombin times and partial thromboplastin times are done on each sample and recorded. Then the above tests are repeated, using fortified plasma (half plasma and half deprothrombinized plasma), and the results are recorded for comparison with the first results. Lee-White clotting times are also done, as well as fibrinogen determinations and serum prothrombin consumption times. A combination of such data from each surgery performed may be the key to solving the various problems that arise in this type of surgery. As I stated previously, the reason for hemorrhage is still unsolved. Blood loss, occasionally leading to death, following this type of surgery may be due to the extreme reduction in body temperature; or to changes in the peripheral blood—causing a reduction in platelets at low temperatures, with failure to increase with the subsequent temperature increase. Or, hemorrhage may result solely from the surgical procedure which is carried out.

Types of heart surgery, other than by hypothermia, have been attempted. One type is a cross-circulation method in which the

lungs and heart of one subject support life in a second, while the latter is being subjected to an operative procedure on his excluded heart.[6] This technique has its drawbacks—yet it allows a longer operating time than does hypothermia. A procedure which makes use of a mechanical heart-lung has also been used; however, it has not been developed sufficiently for use at the present time.[6]

This "frontier" of cardiopulmonary work is still in its "groundbreaking" stage, yet it is destined to become an important area in the profession of medical technology.

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ABSTRACTS

ACID-BASE TERMINOLOGY

Thomas P. Nash, Jr. (University of Tennessee, Memphis), Scientific Monthly 82, 255-7 (1956)

A consistent and precisely defined termniology is necessary when discussion concerning the alkaline reserve and acid base of biological systems is undertaken. These two factors may vary independently of each other—even in opposite directions—and cannot be described by the same terms. The term *alkaline reserve* should be applied to normal, acidosis and alkalosis referring to the CO-2 capacity of venous plasma. The term *acid base balance* should designate the molecular ratio between bicarbonate and carbonic acid components of venous blood plasma and should be expressed as pH of the plasma. Two terms are suggested, acidemia and alkalemia, to describe the acid-base balance when the pH is numerically smaller or larger than normal (7.35-7.45).

THE MICROSPECTROPHOTOMETRIC DETERMINATION OF BLOOD OXYGEN SATURATION IN INFANTS AND CHILDREN

Miriam M. Pennoyer et al (Washington University School of Medicine). J. Pediat. 50, 44-8 (1957)

A micrometric application of the spectrophotometric method of Roos and Rich for the determination of oxyhemoglobin satuation is described. The method includes the blood collection, the spectrophotometric analysis and the initial determination of the extinction coefficients.

SIMPLIFIED RAPID TECHNIC FOR THE EXTRACTION AND DETERMINATION OF SERUM CHOLESTEROL WITHOUT SAPONIFICATION

Julius J. Carr and I. J. Drekter. (Mt. Sinai Hospital and Drekter-Heisler Medical Laboratories, New York, N.Y.). Clin. Chem. 2, 353-68 (1956)

A simple, rapid and accurate method for examining a fairly large number of specimens in a single series has been developed. Cholesterol is extracted and protein removed by addition of acetic acid and acetic anhydride. Acid-catlyzed hydrolysis of acetic anhydride removes the serum water. Development of the Liebermann-Burchard color is then developed with a stable, modified reagent, consisting of equal volumes of sulfuric and acetic acids. Color development takes place in ordinary room lighting without loss of accuracy.

Photometers which show conformity to Beer's law for this color reaction are employed (e.g., Coleman or Klett).

SOME CLINICAL ASPECTS OF LIVER FUNCTION TESTS

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In discussing the subject of liver function tests with medical students and student nurses it has occurred to me that they present something of a special problem. Since these tests can yield information of inestimable value, it would appear that every effort should be made to render a proper understanding of them possible. It has also occurred to me that the technologist is in the peculiar position of understanding the technical difficulties entailed in the performance of these tests; while at the same time he has knowledge of the meaning and interpretations of the results obtained.

My purpose is not to present a critique of the technical aspects of liver function tests because my understanding of this subject may be somewhat less than yours. It is, rather, to help you in an understanding of how the clinician applies these tests in studying a case of liver disease.

It is well to remember that the liver is an organ of very complex action. It is estimated that the liver takes part in over five hundred functions in the course of its daily routine. I suppose many have felt at one time or another there must be at least one test for each of the functions.

Reference to Table I will show you a partial list of the tests which are generally available at the present time for the study of the function of the liver. Many of these are of limited value and are not routinely employed in the study of liver disease. However, there are a few which are employed routinely and which in many hospitals are incorporated into a battery of tests called the liver profile.

It should be remarked that the liver, besides being an organ of multiple functions, is amazing in other ways. For instance, we know that a large portion of the liver may be destroyed by disease process or removed by surgery. The remaining portion of the liver which is intact will continue to carry on the work and function in a perfectly normal manner. It has been estimated that probably eighty or ninety per cent of the liver must be destroyed before it is unable to maintain its work load. One can see from this that in most instances a considerable involvement of the liver must be present before changes in the function tests will be reflected. It is true, too, that once the liver has been damaged it has the capacity to demonstrate a remarkable regenerative power, an ability to heal itself.

At this point let us consider some of the disease processes which we may be attempting to differentiate by the employment

TABLE I

A Partial List of Generally Available Liver Function Tests

Fasting Blood Sugar
Glucose Tolerance Test
Epinephrine Tolerance Test
Fructose Tolerance Test
Galactose Tolerance Test
Blood Lactic Acid
Lactic Acid Tolerance Test
Blood Amino Acids
Blood Urea Nitrogen
Blood Uric Acid
Blood Guanidine
Plasma Fibrinogen
Serum Albumin
Serum Globulin
Takata-Ara Test (Mercuric Chloride Reaction)
Colloidal Gold Reaction
Cephalin-Cholesterol Flocculation Test (Hanger Test)
Plasma Prothrobin
Response to Vitamin K
Plasma Cholesterol—total and fractional
Serum Bilirubin (van den Bergh Test)
Icteric Index
Bilirubin in the Urine
Urobilingen in Urine and Feces
Bilirubin Tolerance Test
Coproporphyrin in Urine and Feces
Bile Salts in Bile
Hippuric Acid Synthesis
Bromsulfalein Test
Rose Bengal Test
Serum Alkaline Phosphatase
Blood Amylase
Plasma Vitamin A and Carotine
Zinc Sulfate Turbidity
Thymol Turbidity
Serum Cholinesterase
Liver Biopsy
Colloidal Red Test

of liver function tests. Table II will serve to orient you in regard to this. Now, of course, not every patient with liver disease is jaundiced. However, a very good percentage of them are.

Jaundice always deserves explanation. It is a spectacular symptom and one which is obvious to anyone observing the patient. It indicates a serious disturbance in that patient's physiology.

For practical purposes it is well for us to think that the clinician is primarily interested in differentiating between jaundice, which may be treated in a medical manner, and jaundice which must be treated in a surgical manner.

This differentiation is extremely important because patients with medical jaundice, if mistakenly operated upon, tolerate the surgical procedure very poorly and are, indeed, poor surgical risks. On the other hand patients with so-called surgical jaun-

dice, will be done a great disfavor if treated with bed rest, diet and medication when surgery is the treatment of choice. In these patients very serious damage to the liver may result if surgical intervention is not carried out within a reasonable time.

TABLE II
A Simplified Classification of Jaundice

I. **Hepatocellular:** "Medical Jaundice." Injury to liver cells. Decreased removal of bilirubin from the blood due to damage to liver cells. Direct van den Bergh reaction positive after very early stage. Flocculation tests positive.
   Examples: Infectious or virus hepatitis. Hepatitis due to infection. Cirrhosis of the liver. Poisoning or toxic effects of drugs such as sulfas, chloroform, carbon tetrachloride, phosphorus, etc.
   Treatment: Bed rest, diet, medication.
II. **Obstructive:** "Surgical Jaundice." Obstruction to flow of bile in ducts leading out of the gall bladder and liver. Positive direct van den Bergh reaction.
   Examples: Stones in the bile ducts. Cancer of the pancreas, bile ducts or intestine. Pressure on the ducts by enlarged lymph glands.
   Treatment: Operation.
III. **Hemolytic:** Due to excessive destruction of red blood cells. Liver function tests are normal. Indirect reacting bilirubin increase.

It may be well to recall that probably sixty to seventy per cent of all cases which come into the hospital with jaundice may be diagnosed with accuracy at the bedside of the patient without the employment of special and complicated liver function or other laboratory tests. When the technical skill of the medical technologist is added to that of the clinician through the use of liver function tests, the percentage of accurate diagnoses in cases of jaundice probably lies around ninety per cent.

Now the multiple functions of the liver which have been previously hinted at may be broadly classified into five categories. Table III illustrates these categories.

TABLE III
General Categories of Liver Function

I. Carbohydrate Metabolism
II. Protein Metabolism
III. Lipid or Fat Metabolism
IV. Pigment Metabolism
V. Detoxication and Conjugation Functions

First, there are the functions which have to do with the metabolism of the three main constituents of our food: the carbohydrate, proteins, and lipid, or fat metabolism. Then there is the pigment metabolism of the liver, and finally the detoxication and conjugation functions of the liver.

From a practical standpoint the information which we expect to obtain from the liver function tests is to differentiate a medical disease of the liver from a surgical disease of the liver. This definition reduces the clinician's problem to its simplest possible

terms. In considering the carbohydrate metabolism of the liver, we will find that the only practical liver function test is the galactose tolerance test. This test will be abnormal in those cases in which damage to the cells of the liver has occurred; such as in infectious or virus hepatitis or in cirrhosis of the liver. The cellular damage is of sufficient degree that the liver is unable to metabolize a dose of galactose which is given to the patient. An abnormal quantity of galactose will then be excreted in the urine and the test will be said to be positive.

Now this is in contradistinction to cases in which some obstructive process involving the ducts leading out of the liver or the gall bladder is present, but in which the liver cells, themselves, are relatively uninvolved. It thus yields a great deal of information in differentiating between those cases due to infection in the liver and those due to obstruction in the ducts leading out of the liver or gall bladder.

Disturbances in the ability of the liver to metabolize proteins may be more easily investigated. The liver is the site of formation of fibrinogen and of albumin. When disease is present, the liver is the site of origin of certain abnormal globulins. Tests for these elements in the blood are generally available. Low levels for fibrinogen are found in those diseases in which liver cell damage is quite extensive. It would be found thus in cases of hepatitis or cirrhosis or toxic effects on the cells of the liver in opposition to those conditions which are due to an obstructive process in the ducts of the liver. The same may be said for the serum albumin of which a moderate to marked reduction occurs in those diseases which give rise to damage to the liver cells. On the other hand the liver is frequently the site of formation of increased amounts of serum globulin or of the formation of abnormal globulins in the blood. These may be studied in detail by the use of electrophoresis and a specific globulin identified. It may be stated further that inflammatory changes are usually necessary for the changes in the serum globulin.

Many of these abnormal globulins will show specific reactions by being precipitable upon the addition of specific agents to the blood serum. For instance, the mercuric chloride reaction or Takata-Ara test demonstrates the presence of an abnormal globulin upon the addition of a mercuric chloride solution to the patient's serum. This test is positive in the presence of cirrhosis of the liver or of other disease giving rise to inflammatory changes in the liver itself.

There are many other turbidity or flocculation tests employing the addition of substances, as widely diversified as thymol, zinc sulfate, or cephalin cholesterol emulsions. In studying diseases of the liver most clinicians will employ the use of several of these tests. The most commonly used are the thymol turbid-

ity, the zinc sulfate flocculation, and the cephalin cholesterol flocculation tests. Each one of these tests, when positive, has specific significance in differentiating one disease of the liver from another. The cephalin cholesterol flocculation test is the one most widely available. Despite the fact that a standard procedure is maintained in doing this test the results from various laboratories will vary. Much of the technical difficulty which is encountered with performance of this test depends upon the fact that certain stabilizing factors are present in the blood of normal patients which will normally prevent flocculation from occurring.[1] In cases of liver disease this stabilizing factor is modified or changed in such a manner that a positive test will result. We do know that the stabilizing factor is subject to change on storage or on heating, so that delay in performance of the test or undue changes in environmental temperature in which the test is performed may result in inaccurate and variable results from any one given laboratory. From a practical standpoint it may be said that if the test shows one plus flocculation at the end of twenty-four hours, a normal result will be obtained at the end of forty-eight hours.

The plasma prothrombin test may be utilized in studying liver function.

However, this is a test which yields positive results usually late in the course of liver disease. Prothrombin is a pseudo globulin which is normally present in the blood and the origin of which is the liver. A deficiency of prothrombin in the blood will give rise to hemorrhagic tendencies.

The principal test employed in studying the lipid or fat metabolism of the liver is that of the cholesterol level of the blood. As you know cholesterol is synthesized in the liver and in the mucosa of the intestinal wall. Cholesterol occurs in both the free and esterified forms in the blood stream. This is of importance because the ratio between the total and esterified cholesterol is maintained at a fairly constant level. In those cases of obstructive jaundice in which obstruction in the ducts is present, there will be an increase in the serum cholesterol accompanied by some increase in the cholesterol esters. On the other hand in those cases with damage to the cells of the liver, such as occurs in hepatitis or cirrhosis, there will be a lowering of the cholesterol due principally to a lowering of the esterified portion. Normally sixty to eighty per cent of the cholesterol in the blood is in the esterified form. However, in those cases of severe damage to the cells of the liver the cholesterol esters will be found to be less than sixty per cent of the total cholesterol and the total cholesterol will be something less than 150 milligrams per cent. Usually this change in the cholesterol due to liver cell damage will occur rather late and after severe damage is present.

This brings us up to a discussion of the pigment metabolism of the liver. From the standpoint of studying diseases of the liver this particular function is one of the most important. In the normal person the serum bilirubin is a pigment derived from the normal breakdown of hemoglobin from the red blood cells. This breakdown of hemoglobin takes place at a rather constant rate. Therefore, the bilirubin will be maintained at a fairly constant level. Normally there is no more than one milligram of bilirubin per 100 cc. of serum. Normally, also, the bulk of this bilirubin is the so-called indirect reacting. The direct reacting fraction constitutes no more than 0.25 milligrams per 100 cc. and usually less than this. These terms derive from the reaction of bilirubin when exposed to Ehrlich's diazo reagent in the van den Bergh reaction. The direct reacting bilirubin is bilirubin which has passed through the cells of the liver; while indirect reacting is the breakdown product of hemoglobin before passage through the liver cells.

The cycle of pigment metabolism in the normal person continues further with the excretion of bilirubin in the bile which is emptied into the intestinal tract. Normally, certain chemical reactions and oxidation take place in the passage through the intestinal tract, converting the bilirubin into urobilinogen and urobilin. Urobilin and urobilinogen will then be excreted in the stools at again a constant and normal rate. The urobilinogen may be measured accurately; and in a twenty-four hour sample of stool it will be found to vary between 40 and 280 milligrams. A small amount of urobilinogen is normally absorbed from the intestinal tract and excreted through the kidneys in the urine. This likewise occurs at a constant and set rate. This excretion may be measured qualitatively or quantitatively by either Ehrlich's or Watson's method. It will be found that between one to four milligrams of urobilinogen is excreted daily in the urine.

Any disease process which affects the liver cells will increase the amount of bilirubin which finds its way into the blood stream. This will give rise to an increase in the serum bilirubin both of the direct and the indirect reacting types. This will likewise cause an increase in the amount of urobilinogen which is present in the intestinal tract and which is eliminated in the urine. Measurements of the urobilinogen contents of the feces and of the urine will then show the degree of liver disturbance which is present. Likewise, there may be an excretion of bilirubin into the urine. Any disease process resulting in an obstruction of the ducts leading out of the liver, will, of course, give rise to an increase in the bilirubin in the blood. This increase will be more marked in the direct reacting bilirubin because the obstruction in the flow of the bile takes place after the bile has passed through the liver. A serum bilirubin showing a high de-

gree of increase in the direct reacting bilirubin will, therefore, be more indicative of an obstruction than of a disease process affecting the liver cells primarily.

The principal test employed in many laboratories for a number of years for the study of pigment metabolism of the liver has been the icterus index test. This has yielded notoriously variable results through no fault of the technologist.

Many clinicians have come to distrust the results obtained with this test and for rather just reasons too. Technically the test is fraught with difficulties. A small amount of hemolysis obtained at venipuncture, despite the greatest care will materially affect the result obtained with the test. This hemolysis may be so slight as not to be visible to the naked eye. Also turbidity of the serum as a result of the postabsorptive state will affect the results obtained. There are certain other pigments in the blood which may normally impart a yellow tinge to the serum and which will interfere with the results of the test. These pigments for the most part are carotene and are normally found in carrots, oranges, peaches and other yellow pigmented fruits and vegetables. The serum of many people who eat generously of these particular foods will have the yellow color imparted by carotene. Most laboratories use six units as the upper limit of normal for the icterus index. However, rarely will results below eight or ten be obtainable with consistency. Many clinicians will allow a result of eight or ten units to be considered normal for this test.

There is a correlation between the results of the icterus index and the serum bilirubin. The icterus index will be ten times the value of the serum bilirubin. This is true at low levels of these tests and may not be true when higher levels are obtained.[2]

A recent report[3] by Henry et al. demonstrated a method which may eliminate many of the inaccuracies obtained with the icterus index.

This procedure involves an acetone extraction of the serum in an effort to remove proteins including hemoglobin left there by hemolysis. It also removes lipids from absorption of foods previously eaten. The filtrate obtained from the acetone extraction is read in the spectrophotometer at a different absorption band than is the usual, again, to avoid confusion with hemoglobin.

There are two very valuable tests employed in studying the detoxification and conjugation functions of the liver. One of these is the hippuric acid synthesis test which depends upon the ability of the liver to form hippuric acid upon administration of a dose of sodium benzoate. This test will serve to differentiate, again, medical from surgical jaundice and has a great deal of value in questionable and borderline cases.

The bromsulfalein test is a very valuable test, particularly in

detecting complete recovery from certain types of medical jaundice and also in the early diagnosis of cases of cirrhosis of the liver. The bromsulfalein test is an extremely sensitive indication of the functioning ability of the liver cells. As a general rule the test will be abnormal in those cases in which the serum bilirubin is elevated. However, it has one other valuable aspect. It will yield abnormal results at times when the serum bilirubin has returned entirely to normal or at times before the disease process is extensive enough to cause elevation of the serum bilirubin.

As you know, there are two procedures for the bromsulfalein test; one employing a two milligram per kilogram dose of the dye, the other employing a five milligram per kilogram dose of the dye. I have found that the five milligram dose is more valuable because it imposes a greater burden on the liver and will theoretically yield abnormal results in cases of milder functional impairment. A note of caution is in order in regard to this test. The dye is a very irritating substance when injected outside of the vein. It may be the source of considerable pain and discomfort in the event of this occurrence. It is true, further, that the deposition of the dye outside the vein will materially affect the results of the test obtained. A considerable amount of credence is placed upon the results of the test; and a great deal of damage and harm may be done to the patient whose result is abnormal by virtue of the fact that the dye has not been properly injected. In any case where a small amount of dye escapes outside the vein, it is best to discontinue the test and to report this to the clinician so that it may be repeated at a later date. I have witnessed a severe reaction to injected bromsulfalein in which an allergic response occurred. This has been reported previously but fortunately is very rare.

Another test of liver function which has been found to be of value is the alkaline phosphatase level. The origin of alkaline phosphatase normally present in blood serum is known to be the bone marrow. However, the actual transport of the phosphatase between the bone marrow and the liver and the methods of excretion by the liver are not definitely known. It is well established that any condition resulting in obstruction to the flow of the bile in the ducts leading out of the liver will lead to a marked increase in the value for the alkaline phosphatase. The various methods employed to determine the alkaline phosphatase will not be confusing as long as the method employed is expressed with the result obtained.

Recently the plasma cholinesterase activity has been employed as a test of liver function. Again, this is a test of the presence of a specific enzyme in the plasma. It will be found to yield abnormal results in cases in which liver cell damage has resulted. Therefore, it will be positive in cases of hepatitis or cirrhosis,

but is unlikely to be positive in cases of obstruction to the ducts or the gall bladder or liver. A rather specialized technique is necessary for the performance of this test; and it is, therefore, not generally available at the present time. However, it is hoped that simplified methods will be found for its performance. In general, it may be said the test will be found to be positive in those cases where the flocculation or turbidity tests are positive. However, it is of more value than the flocculation tests in cases of convalescing hepatitis or in cases of chronic liver disease, such as cirrhosis in which the flocculation tests are sometimes negative.

In the preceding discussion I have outlined a number of tests which may be employed in studying diseases of the liver. I don't believe that any clinician will routinely employ all of the tests mentioned above; but in the initial study of the patient, most physicians will limit themselves to a certain battery of tests which have proved to be the most revealing. Most information may be obtained in studying a given case of liver disease by limiting oneself to first, determination of the serum bilirubin, both the one minute reacting and the total; secondly, the determination of the A/G ratio; third, the employment of the flocculation tests, usually the cephalin cholesterol flocculation and the thymol turbidity; fourth, determination of the total cholesterol together with the cholesterol esters; fifth, the alkaline phosphatase; and sixth, investigation of the excretion of urobilinogen in the urine.

When these tests yield normal results or indefinite information, one may resort to the use of further function tests when necessary.

It is well to bear in mind that repeated testing at intervals of several days to a week will frequently reveal considerable information. During the early phases of a disease process some of the tests may not be positive, but over a period of a week, they will become so and the diagnosis may become obvious on further study. In most cases of jaundice it is frequently well, and without harm to the patient, to employ several days to a week for the study of the case with repeated liver function tests. As intimated above it is of extreme importance to differentiate a case of medical from surgical jaundice. Confusion of the two may result in great harm to the patient.

It is most reassuring to have laboratory corroboration of that judgment at times when reasonable doubt exists.

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HAZARDS ASSOCIATED WITH THE HANDLING OF PATHOGENIC BACTERIA*

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Most of us know of some fellow technician who has contracted a communicable disease as a direct result of his work. Without making any effort to hunt for them, I myself have come to know of some thirteen cases of tuberculosis acquired by technicians in my province, in the course of their work. Some of these were clear cut cases, such as self-inoculation. I know of two different technicians who did this while inoculating guinea pigs with tubercle bacilli. Both developed clinical tuberculosis. Then there was another startling case. A worker was inoculating guinea pigs, again with a live suspension of virulent tubercle bacilli. Wishing to get rid of an air bubble in the syringe he absent-mindedly raised the needle and pressed the plunger. The resultant spray infected two assistants who were in his line of fire.—Four months later they developed pulmonary tuberculosis. Not all cases are so clear cut. There was a young fellow who washed glassware and tended the animals who contracted tuberculosis. He could have gotten his infection on the street or anywhere, of course, but examination of his work surroundings showed several dangerous areas. In the animal room a guinea pig occasionally developed open, discharging tuberculous lesions; in his sink incompletely decontaminated glassware from the bacteriology laboratory found its way; in the autopsy room where he had to help occasionally, there was much to be desired in the way of cleanliness.

There is an element of hazard associated with certain phases of laboratory work. It is associated particularly with the culture and animal studies of any highly pathogenic microorganism. It differs from the hazards associated with, say, flying or electrical work in that the consequences of faulty practices, faulty equipment or just plain mistakes are seldom as dramatic or sudden. But in the last analysis they may be as deadly.

A year ago the Canadian Society of Laboratory Technologists set up a committee to study this problem. The disturbingly large number of technicians in Ontario, who had contracted tuberculosis over the past ten years had been noted. Recognizing also that the tubercle bacillus is now being cultured, subcultured and pipetted as never before, it was felt that a study of the occurrence of tuberculosis among technicians should give us a clue to the extent of these infections. Accordingly, informa-

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tion on tuberculosis morbidity among Canadian technicians was obtained through Provincial channels. Though the information obtained is far from complete, it demonstrates that the incidence of tuberculosis among the group studied is higher than among the general population.

TABLE 1
Recorded Cases of Tuberculosis Amongst Medical Laboratory Technicians in Canada

Province	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	Total
B.C.			0	0	0	0	3		1	0	1	0		5
Alta.	1	0	0	0	0	2	4	1	0	0	0	0		8
Sask.		3	0	0	0	1	0	0	0	1	0	2		7
Ont.											*16			16
N.B.								0	0	0	0	0		
N.S.							0	0	0	0	0	0		
P.E.I.		0	0	0	0	0		1	0			3		4
N.F.			1										1	2
	1	3	1	0	0	3	9		1		23		1	42

* There were 18, but 2 were left out because they were ex-patients.
This table was compiled from information obtained from the Departments of Health of each Province.
Blocked out areas refer to those covered in Table 2.

TABLE 2
Tuberculosis Morbidity Amongst Medical Laboratory Technicians Compared With That in the General Population

Area	Period	Group	Individuals Involved	Cases	Case Rate[a]	Ratio
ONTARIO	1952-1954	Technicians	800[b]	16[c]	667	28:1
	1952-1953	General Population[e]	4,897,000	2,699	28	
CANADA—less Que. and Man.	1947-1954	Technicians	1,400[b]	36[c]	581[d]	8.4:1
CANADA—less Que.	1947-1953	General Population[e]	10,487,000	50,496	69	

(a) Annual incidence per 100,000.
(b) Estimated number of technicians working in this area.
(c) See Table 1.
(d) Arrived at from the case rate per province per year for the period covered.
(e) Morbidity figures for the general population are from Drolet and Lowel, Amer. Rev. of TB., 72, 428 (Oct., 1955).

It is realized that this comparison of tuberculosis incidence rates in technicians with those in the general population is open to criticism on the grounds that: (1) Tuberculosis may be searched for and reported more thoroughly in hospital and laboratory staff than in the general population. (2) The number of cases reported annually is small and apt to fluctuate widely. (3) The age distribution of the laboratory technician population differs from that of the general population. Nevertheless it is significant that on applying the Ontario case rate (Drolet and Lowel) for the three years, 1951-1953, to the approximately 800 technicians in Ontario, one would expect no more than one new case during this period. This estimated figure is significantly less than the recorded figure of 16 cases.

Acknowledgment is made to Dr. E. N. McKay, Div. of Medical Statistics, Dept. of Health of Ontario, for her critical assistance in evaluating these figures, and also Dr. J. W. Bell, Div. of Laboratories, for his valuable advice in preparing the tables.

What about infections other than tuberculosis? The literature is full of reports of other laboratory-acquired bacterial infections, and virus and fungus infections also. Certain organisms, such as *Brucella* and *Pasteurella tularensis* and *Coccidioides immitis* are more dangerous to handle than *M. tuberculosis*. One Canadian laboratory traces five infections with *Brucella abortus* and one with *Pasteurella tularensis* among its small staff, to the preparation of antigens with these organisms; and I call to mind, too, the technician who contracted a dysentery which was subsequently found to be caused by a rare strain of *Shigella*, which she had recently pipetted.

All right, then, technicians get infected. How does this happen? What can be done?

Basically, there are three routes of infections: ingestion, inoculation and inhalation. Of the first two, little should need be said since the answers are fairly obvious. Common sense should prevent one from smoking or eating in infectious areas. The wearing of protective clothing, keeping the hands away from the face, and a thorough clean-up afterwards should be an automatic part of any procedure involving the transfer of dangerous microorganisms. The danger of self-inoculation with a capillary pipette may be completely overcome by substituting the blunt type for the conventional sharp-tipped Pasteur pipette whenever pathogenic organisms are handled. And in using a syringe, the animal-holding hand should never be in front of the needle. I repeat: the hand which does not hold the syringe must be kept back of the needle point at all times. Any technique which calls for holding in front of the needle point is faulty and needs revising even though the material is avirulent. And don't point loaded syringes!

The danger of infection by inhalation of aerosols and other airborne particles is more difficult to see, and about this I should like to speak at greater length. Everyone can see a splash or spill and do something about it. But the aerosol is a sports car of a different colour. It has been shown by Dr. Wedum and his workers at Camp Detrick, that, in the ordinary techniques of mixing and transferring fluids, mists or sprays made up of extremely small particles get out into the air. Here is a list of common laboratory procedures in which it has been proved that these aerosols are given off:

Mixing of fluids by shaking.

Mixing fluids by bubbling or squirting with a pipette.

Blowing the last drop out of a pipette. (The capillary pipette is specially dangerous here).

Removing a wet cotton plug or other stopper from a tube or bottle.

The impact of a falling drop on any hard surface, or even on dry cloth.

Immersion of a hot loop or needle in a fluid.

Touching a loaded inoculating needle to the side of the tube, causing it to vibrate.

If we keep in mind that the particles in an aerosol are so small that their dried residues tend to remain suspended in the air almost indefinitely, moving freely with every little air current, we can readily see the hazards created by many of our common practices. The answer to this problem of airborne infection, whether due to aerosols or to the spores of pathogenic fungi, seems to be the biological safety cabinet. In this, the operator works with a shield of glass between his face and his work, and a current of air sweeps aerosols away from him through a decontaminating device and out of the building. Note that if the worker referred to earlier who absent-mindedly sprayed his assistants had been working in such a cabinet no harm could have resulted. Dr. Wedum calls the Safety Cabinet the most important single item of equipment to safeguard those working with highly pathogenic microorganisms. I feel that its use in the handling of dangerous organisms is as important to the technician as is the guard on his power saw to a carpenter, or the heavy insulation on his high-voltage equipment to the x-ray technician. I am convinced that the bacteriological laboratory of the future will be built around such safety devices, and that highly dangerous work involving the transfer of cultures, as well as animal inoculation and autopsy, will no longer be tolerated on open benches.

Now, what can we do? What can each one do? Each one can and should critically examine his techniques and working conditions and re-examine them frequently. If they need improving, improve them. He should remember that not only his own safety but the safety of his fellow-workers is at stake. For those who are instructing students, we should beg that they emphasize this aspect. For those who purchase or influence the purchase of laboratory equipment and supplies, we recommend that careful consideration be given to the safety aspect. For those who may be designing or helping design a new laboratory, we make the observation that safety considerations should influence design. Our Safety Committee hopes to develop a booklet on safety techniques and equipment and will appreciate any constructive suggestions. Let us be safety wise and make others the same. As Dr. Wedum says, "We take a well justified pride in our martyrs to public health research—those who have sacrificed their lives to save others from disease—but the time has come for us to take an equal pride in efforts to prevent such martyrdom."

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THE ROLE OF 'O' Rh NEGATIVE BLOOD IN THE EMERGENCY BLOOD BANK*

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The use of the so-called "universal" donor has been deplored by numerous authorities (9, 10, 19) who have pointed out the many dangers involved in the indiscriminate usage of blood from such donors. The key word here is "indiscriminate."

On October 29, 1947, at our hospital, an emergency shelf was added to our Blood Bank. On this shelf was placed blood that could be given to any patient on immediate demand, without prior cross-matching, if the patient's condition so warranted. This was blood that fulfilled the following requirements: was Group 'O', cde/cde, Du negative, had a titer of saline and immune Anti-A and Anti-B antibodies below 1:200, and did not contain antibodies against the 'D' factor.

From that day in 1947 to the present, March, 1956, we have used 253 bottles of blood from this emergency shelf without a single fatality, without a single severe reaction; in fact, without a single transfusion reaction of any kind.

Most errors in blood transfusions occur because of the mistyping of blood. It has been claimed that there is an 8-9% error in blood typing, mainly due to the use of unqualified persons. Wiener has stated unequivocally that blood typing tests must be carried out by trained technologists and not by untrained internes or other unqualified persons.[1]

The physician and surgeon who is in a position to use his judgment when evaluating the result of any other laboratory test is completely at the mercy of the technician when given a bottle of blood with the statement that it is compatible and safe for his patient.[19]

So we feel that by the use of properly trained persons, i.e., registered medical technologists, and by the performance of certain tests, the calculated risk of giving blood, even blood without a Cross-match, can be greatly minimized. But we reiterate, this blood is to be given only in an emergency, to patients who are so critical that even a delay of minutes may be vital.

Let us take these tests in detail. Since they are performed in advance and at leisure, they can be quite extensive. We use a printed form as a record and to avoid omitting any tests.

The ABO grouping.[17] The typing of the cells is done by the slide method, using a 40% cell suspension in their own serum and the appropriate Anti-A and Anti-B typing sera. (All serum used meets N.I.H.[4] requirements.) This test is done at room

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Donor No.	A B C			RH				A & B Titre	Im'une A & B	RH Antibody
	Slide	Tube	Serum	C	D	E	Du			

SAINT MARY'S HOSPITAL LABORATORY

temperature, avoiding heat, for A and B antibodies have optimum activity at 20° C.[3] The test tube method of typing, using a fresh 2% saline cell suspension is also done, by a different medical technologist. Place one drop of Anti-A serum in a test tube (the 10 x 75 mm. size is used) labeled 'A' and one drop of Anti-B serum in a test tube labeled 'B'. Add one drop of the 2% saline cell suspension to each tube. The tubes are well mixed and centrifuged for two minutes at 1000 rpm. The presence of agglutination is observed macroscopically.

The next phase is the serum typing. The donor's serum is inactivated for 10 minutes at 56° C. in a water bath, and then cooled to room temperature. A fresh 2% saline suspension of known 'A' and known 'B' cells are prepared. (The 'A' is a pooled sample, representing at least three different 'A's.) Then the serum of the donor is set up against these known cells in small test tubes, mixed well, centrifuged, and read.[3,17]

For the Rh typing, the slide method is used.[17] First the donor's cells are tested against Anti-D; if negative, against Anti-CD and Anti-DE. If negative to all three typing sera, it is presumed to be cde/cde. Be careful to watch for rouleaux formation, and the drying up of the mixture which may be interpreted as a positive reaction. Do not read microscopically.

Since 2-3% of all D's are positive for Du,[4] we must check for this factor. The cells of the donor (in a 2% saline suspension) are sensitized with immune Anti-D serum by placing 2 drops of Anti-D serum and 2 drops of the donor's cells in a small test tube. The mixture is incubated for 30 minutes in a 37° C. water bath, washed three times with saline, and a Coombs test is done.[6]

A note on the Du factor. Donors who are positive for Du are handled as other D's, for Du is an antigenic as D. Patients who are positive for Du are treated as d, and given blood negative for D. Anti-D antibody formed by a Du patient has been reported.[5]

The last steps are the checking of the donor's serum.

The presence of Anti-D antibodies is established by testing the serum of the donor (2 drops), against pooled type 'O' known

D's in a 2% saline suspension (2 drops) in a small test tube and incubating for 60 minutes at 37° C. If negative, the mixture is washed three times with saline and a Coombs test is done.

For the detection of the saline Anti-A and Anti-B antibodies, the donor's serum is first diluted 1:100 with saline. Then this diluted serum is tested against known 'A' (pooled) and 'B' cells as in the saline tube typing, making a final dilution of 1:200. 'O' blood can be safely given to heterologous recipients when the serum of Group 'O' blood has a titer of saline Anti-A and Anti-B iso-agglutinins below 1:200.[8, 19]

But the saline agglutination titer, though below 1:200, is not in itself reliable for safety.[7, 13] The immune antibodies must be investigated. So, if there is no clumping in the preceding tests, we now check for immune Anti-A and Anti-B antibodies. The above tubes are incubated for 30 minutes at 37° C., washed three times with saline, and Coombs serum is added. Immune Anti-A and Anti-B antibodies can be detected satisfactorily with anti-globulin serum,[6, 7, 9, 13] but the reaction is not as strong as for Rh antibodies, so that great care must be taken in the performance and interpretation of this test.

These Coombs tests for the Anti-D and the immune Anti-A and B antibodies are also of value in that they may pick up antibodies against the other rarer blood groups, such as Kell, Duffy and Kidd.[18]

The use of the blood group specific substances A and B, has little or no effect on immune Anti-A and Anti-B.[11, 12] Therefore, their use gives a false sense of security and they should not be used.

There is another danger in the use of the blood group specific substances. A group 'O' recipient of such blood may develop immune type antibodies that would render the plasma of such an individual dangerous for use at a subsequent period.[14, 15] In cases of female recipients, this procedure may conceivably lead to hemolytic disease of the newborn when an ABO incompatibility exists.[14, 19]

The screening of donors is important. A donor having had a recent injection of horse serum may produce a rise in Anti-A immune antibodies. A recent pregnancy or injection of bacterial vaccines may also produce the same condition.[7]

Two other tests were tried by our blood bank and discarded. One, the Witebsky neutralization test[9, 11] would merely have been a repetition of our other tests and therefore, unnecessary. The other, the hemolysin test, has been recently advocated[14] by several workers. But we agree with Zuelzer[16] and found it too sensitive.

The giving of any blood to any patient involves some degree of risk and every precaution possible should be taken to mini-

mize that risk.

It must always be remembered that a mistake in blood bank procedures is invariably fatal. However, with qualified personnel performing a complete battery of tests, the so-called universal donor can be used safely. At our hospital this blood is used only in a dire emergency. And because it was available, it has saved many lives.

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THE PREPARATION OF LARGE TISSUE SECTIONS FOR MACROSCOPIC STUDY*

ROY J. WILKINSON, R.T. (Hist.), Senior Histologist,
Sunnybrook Hospital, Toronto, Ont.

In 1949 Gough and Wentworth, working at the Welsh National School of Medicine, Cardiff, Wales, described a method for the study of the pathological anatomy of the lung in coal-miner's pneumoconiosis. Since that time many workers have followed their technique and adapted it to other organs of the body with acceptable results. The method entails the cutting of gelatin-embedded, frozen sections of an entire organ by means of a large sliding microtome and mounting them unstained on filter paper. The technique I am about to describe is basically that of Gough and Wentworth, though several modifications have been made which I believe simplifies the procedure and produces a more satisfactory preparation. Although I have had excellent results with some other organs, for the purpose of this paper, I will confine the technique to the preparation of lung sections.

The lungs are removed from the body whole and without rupturing the pleura. If there are dense adhesions, take the parietal pleura out with the lung. Cut off at the hilum and fully distend by running a fixative into the major bronchi, by means of a tube and cannula from a reservoir about 4 feet above the lung. The fixative is 4% sodium acetate in 10% formalin. When the lung is fully distended the bronchus is tied off securely so that the fixative does not escape and the lung is placed in a container of the above fixative large enough for it to float freely with no distortion from pressure. Cover with a cloth wet with the fixative. The amount of fixative necessary to distend the lung varies up to about 2 litres and in the container I use there is a further 3-4 litres.

Fix for 1 week or longer and then cut a slice about 1 inch thick. This cut is made in the most suitable direction for the demonstration desired. It is desirable to have the slice cut at an even thickness throughout its entirety.

Wash the slice in running water for about 72 hours to remove the formalin and place in a 25% aqueous solution of gelatin to which has been added 2% cellosolve and .5% caprylic alcohol. The slice is placed in the gelatin solution at a temperature of 55°-60° C. I find a vacuum dessicator, 250 mm. in diameter, is a satisfactory container for this purpose, because as a partial vacuum is created, the removal of air from the slice is facilitated and this in turn assists the penetration by the gelatin. Sufficient

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956. Published in the Canadian Journal of Medical Technology, Volume 18, No. 4, December, 1956.

air can be removed within an hour, during which time the gelatin remains fluid at ordinary room temperature. The specimen is then kept in this gelatin solution in an incubator at 37° C. for about 72 hours, care being taken that the specimen is completely immersed and the container covered to prevent evaporation.

When impregnation is complete, the specimen is cast in a block by immersing it in melted gelatin in a shallow dish and allowing the gelatin to harden. In casting, it is important that the specimen be kept as flat as possible, especially the surface to be cut. This can be accomplished by weighting the specimen against the bottom of the dish. I find it beneficial to allow the gelatin block to "set" in the refrigerator for about 24 hours. After this time the gelatin is firmly set and enough shrinkage has occurred that it is readily removed from the dish. The block is trimmed and then fixed to the microtome holder by warming the latter and then putting weights on top of the block until the gelatin sets and sticks the block to the holder. The block fixed to the holder is then placed in a freezer at —15° C. overnight or until such time as sections are to be cut.

The microtome used is the large section microtome designed by Gough and Wentworth, manufactured by Measuring and Scientific Equipment Ltd.

When sections are to be cut the microtome is prepared and pans of water at refrigerator temperature are made available. The block frozen to the microtome holder is removed from the freezer and attached to the microtome. Sections are cut as thawing takes place. A warm towel is rubbed on the surface to hasten thawing. Do not cut sections until the block is sufficiently thawed to cut easily. Sections are cut at about 400 microns but it may be necessary to cut thicker or thinner depending on the nature of the specimen. With a block of tissue one inch thick, up to 40 satisfactory sections may be obtained. When cut, sections are transferred to cold water and placed in a refrigerator. Gough recommends placing sections in 10% formalin, for 24-48 hours to harden gelatin and then washing 1-2 hours before mounting. I find this step undesirable when handling a large number of sections because if sections are placed in formalin they require washing for mounting. This has to be done individually which is very time consuming. Also, on occasions, the gelatin becomes too hard and this causes difficulty when mounting. Sections will keep up to 1 week in water at refrigerator temperature. Before attempting to mount sections the excess gelatin is trimmed from around the edges and any loose blood clots are removed from the large blood vessels.

Mounts are made of a uniform size which is 9¼″ x 12″. This size will accommodate the largest specimen which is practicable

to cut. The sections are mounted on Whatman #1 filter paper 11" x 13" which allows for later trimming to the above size. Three sheets of paper are required for each section. The identification and diagnosis of the case are typed on the lower end of the rough side of one of these sheets. The sections are mounted in the following manner. A sheet of "plexiglas" 11" x 13" and $\frac{3}{16}$" thick is carefully washed and dried and the following freshly made mounting medium, which is kept melted in an oven at 55°-60° C. is poured on it and spread to a thin, even layer.

The mounting medium is a 15% aqueous solution of gelatin to which has been added 12% glycerin and 1% of a 10% solution of camphor in methyl alcohol.

The section, which has been brought from the refrigerator and allowed to warm almost to room temperature, is quickly transferred to the "plexiglas" plate. This can be readily accomplished by lifting it at the upper $\frac{2}{3}$ of the section with the ends of the fingers of both hands. The lower $\frac{1}{3}$ of the section is allowed to contact the "plexiglas" and the fingers are rolled out from the upper portion. The wrinkles are teased out by use of a bent glass needle such as used in handling quick sections. If any difficulty is encountered, the addition of more mounting medium will help. Care should be taken to orientate the section on the "plexiglas" in such a manner that one would view the finished mount as one would an X-ray film of the specimen in situ. When the section is properly in place an excess of mounting medium is carefully poured over it. Three sheets of filter paper as prepared above are then applied and further mounting medium is flooded over them. This should be sufficient to saturate them. Air bubbles will be trapped between the sheets of paper but the excess mounting medium makes it possible to roll these out with a 6" rubber print roller. Only the weight of the roller should be used until all air bubbles are out and then light pressure may be applied to remove surplus solution. This preparation is allowed to dry overnight and preferably longer; in any case until it is thoroughly dry. The paper with the section adhered to it and covered with a thin film of gelatin can then be stripped from the "plexiglas." It will be found that the three sheets of paper and the section have become intimately and permanently adherent to each other and appear as one unit.

The mounts are then trimmed to desired size and placed between sheets of cardboard and pressed for 24 hours to alleviate their tendency to curl. Care should be exercised in handling the mounts as fingerprints are readily left on the coating of gelatin. To make a more satisfactory permanent mount these sections are sprayed with a very thin coat of clear plastic. Plastic spray in aerosol cans such as is used to spray the "chrome" on cars has been found satisfactory. The plastic coating is allowed to

dry overnight. Mounts prepared in this manner will not show fingerprints and may be wiped with a damp cloth without causing any damage.

I have described a technique for preparing large sections for macroscopic study. These preparations serve as an excellent means to correlate the findings of the gross anatomy with the microscopic histology. These sections appear like photographs of the cut surface of the organ but show more detail than can be reproduced by photography. They also are a desirable medium for the study of the extent of a lesion and its exact location. They have much to offer over the conventional museum specimen mounted in a jar. Many sections can be produced demonstrating the same lesion. These could be passed around for study at a conference. They may also be viewed along with the X-ray film of the organ and the X-ray shadows compared with the pathologic areas in the organ. They are suitable for mailing and may be filed with the patient's case history as a permanent record.

In conclusion I would like to express my thanks to Dr. A. J. Blanchard, Director of Laboratories, Sunnybrook Hospital, under whose direction this work was carried out, for his valuable assistance and encouragement in the preparation of this paper.

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ABSTRACTS

BLOOD pH MEASUREMENT WITH THE GLASS ELECTRODE STUDY OF VENOUS AND FINGERTIP BLOOD

Samuel Natelson and Norbert Tietz (Rockford Memorial Hospital, Rockford, Ill.). Clin. Chem. 2, 320-7 (1956)

Blood pH drifting toward the alkaline side when exposed to air at 37° was rapid with fingertip blood but slower with venous blood. A slower drift was noted when blood was collected under oil. The pH did not tend to drift when the blood was collected under oil and cooled rapidly to room temperature.

The difference in the drifting to the alkaline side was attributed to the release of carbonic anhydrase being greater in the serum from finger puncture than in that from venipuncture. It was noted that serum from patients in acidemia with low CO_2 content did not drift at 37°.

This study was made by testing the effect of carbonic anhydrase and its inhibitor, Daimox (2-acetylamino-1, 3, 4-thiadiazole-5-sulfonamide) on the drift of pH in the blood and plasma from normal individuals.

In the summary it is advised that blood pH determinations be made by collecting blood under oil, cooling rapidly to room temperature and performing the determination under oil on the separated serum.

PLASMA FIBRINOGEN DETERMINATIONS A RAPID TITRATION METHOD

Arthur A. Rosenberg. (Veterans Administration Hospital, Albany, N.Y.). Clin. Chem. 2, 331-3 (1956)

An oxalated (with potassium oxalate or ammonium-potassium oxalate) blood specimen is centrifuged for five minutes and at least 1.5 ml. plasma is separated. Serial dilutions are made with physiologic saline solution and 1 drop of 0.25% methylene blue added. At about two second intervals 0.2 ml. of thrombin solution is added and the tube shaken gently. The tubes then are allowed to stand at room temperature for five minutes. Then 2 ml. of saline is added to each tube. The highest dilution showing a definite clot is the titer. This is noted easily by the two-hue color phase when there is a clot and an even distribution of methylene blue when there is no clot.

Parallel quantitative determinations of fibrinogen protein nitrogen have shown that a titer of 128 or higher correspond to a fibrinogen content in excess of 200 mg. per 100 ml.; a titer of 64 corresponds to a fibrinogen content of 100-200 mg. per 100 ml.; a titer of 32 or less corresponds to a fibrinogen content under 100 mg. per 100 ml. Abnormal bleeding occurs when a titer of 32 or less is obtained.

ASMT SILVER ANNIVERSARY CONVENTION

Palmer House, Chicago, Illinois

June 23-28, 1957

The skyline of the "Windy City," with its almost never ceasing breezes from Lake Michigan. Chicago, the second largest city in the country and the fourth largest in the world.

THEME: GROWTH THROUGH SERVICE

CHICAGO

The city itself—a pleasant place to visit—to vacation—and for the program we look forward to hearing—

John Ott, Jr., S.D., who will present a talk with time-lapse pictures at the ASMT Silver Anniversary Convention on "Research Through Time-Lapse Photography."

John Ott is a pioneer of time-lapse photography, lecturing and showing his pictures to all types of audiences across the country. He has produced TV films in which he takes part himself in telling the story for such companies as the Atchison, Topeka and Santa Fe Railway, Eastman Kodak Company, Green Giant Company, Kimberly-Clark Corporation, The Quaker Oats Company, Swift and Company, Standard Oil of Indiana, The United Electric Coal Companies and many others.

John Ott first showed his films on TV in the early part of 1949, soon after the first station started in Chicago. Since then he has supplied films or taken part in various TV programs including the network program "Home," "Today," "Zoo Parade," "Out on the Farm" and "Disneyland" in addition to doing his own television show regularly, "How Does Your Garden Grow," which is in its sixth year. The Radio Corporation of America used John Ott's time-lapse pictures to demonstrate color TV to the FCC when approval was won for the RCA Color System, and his garden program was the first to originate in color from Chicago. Walt Disney uses many of John Ott's pictures in such award winning motion picture films as "Nature's Half Acre" and "Secrets of Life."

Carroll L. Birch, M.D., will present a talk at the ASMT Silver Anniversary Convention on "Medical and Social Aspects of India: Based on Three Years' Experience."

Carroll L. Birch, M.D., is Professor of Medicine, University of Illinois, College of Medicine and a Reserve Officer in the United States Public Health, Rating of Medical Director. Her interests and her work have taken her to many countries. She has studied hemophilia in the European royal families; intestinal parasites in Costa Rica; malaria in Tallahassee under the Rockefeller Foundation; bonemarrow in leprosy while visiting Puerto Rico; African sleeping sickness while traveling in Africa for seven months.

Dr. Birch has recently spent three years in India with the United States Department of Health, Education and Welfare as Dean of Lady Harding Medical College, New Delhi. She was the only non-Indian at this medical school and on the compound. She also acted as Superintendent of the hospital and School of Nursing.

Dr. Birch has made two motion pictures; one on "Haemopoetic Principle" and the second on "Bone Marrow." She received the Elizabeth Blackwell Award for outstanding contribution by women to knowledge of medicine at the New York Infirmary in 1956. She was voted the Woman Physician of the Year in 1952.

This last year Dr. Birch visited Norway, Sweden and Russia on her way to the Medical Woman's International Association meeting in Switzerland, stopping in Spain and Portugal on her way home.

Dr. Birch's extensive adventures in travel and study have been recorded on kodachromes and reflected in her many lectures to medical students and to lay and professional groups on hematology, tropical medicine and parasitology.

Dr. Natelson will talk on "Micro Determination of Blood pH: Problems and Errors."

Samuel Natelson, Ph.D., Rockford Memorial Hospital, Rockford, Illinois, is known throughout the country for his work on micro techniques, lecturing and instructing at seminars and state meetings to the medical technologists as well as to other professional groups.

Dr. McGrew will present a talk at the ASMT Silver Anniversary Convention on "Role of the Medical Technologist in Exfoliative Cytology."

Elizabeth A. McGrew, M.D., Associate Professor of Pathology, University of Illinois, College of Medicine and Associate Pathologist, Research and Education Hospitals, University of Illinois, includes among her various projects sex-chromosomal studies. She is a former M.T. (ASCP) of the University of Minnesota.

Dr. Hoffman will talk on "Technical and Theoretical Limitations of Blood Chemistry Determination" at the Silver Anniversary Convention of ASMT.

William S. Hoffman, Ph.D., M.D., is Professorial Lecturer in Medicine, University of Illinois College of Medicine and Medical Director, Sidney Hillman Health Centre of Chicago. For many years he was Acting Director then Director of Biochemistry, Hektoen Institute for Medical Research, Chicago, Illinois. Included among his many publications are "Photelometric Clinical Chemistry," published by Wm. Morrow, New York, 1941, and "Biochemistry of Clinical Medicine," Year Book Publishers, Chicago, 1954.

Dr. Morrill will present a talk at the ASMT Silver Anniversary Convention on "Application of Medical Technology to Animal Diseases."

C. C. Morrill, D.V.M., is at present Professor and Head of the Department of Veterinary Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan. He has been associated with the departments of veterinary pathology at Kansas State College and the University of Illinois. His lectures to lay and professional groups have delighted and enlightened the audiences. The College of Veterinary Medicine at Michigan State gives a curriculum in medical technology and Dr. Morrill's department teaches at least three courses to the medical technology students including a course in general pathology.

John W. Rebuck, M.D., will present a talk on "Structural Changes in Lymphocytes, Monocytes and Plasma Cells in Blood Diseases."

John W. Rebuck, M.D., Ph.D., Senior Associate in Pathology, Henry Ford Hospital, Detroit, is known to the medical profession throughout the country for his numerous lectures and prolific publications. For several years he has been conducting a course on "Bone Marrow Interpretation" for the pathologists and is chairman of that workshop for the American Society of Clinical Pathologists. He is the author of two text books, the first "Leukocytic Functions" published by the New York Academy of Sciences in 1955, and the new comprehensive text book on "The Leukemias" published by the Academic Press in 1957.

SYMPOSIUMS

1. "Progress and Problems on Blood Bank Technology and Immunohematology"

Moderator: Kurt Stern, M.D., Director, Blood Center, Mount Sinai Medical Research Foundation.

Panelists: T. J. Greenwalt, M.D., Medical Director, Milwaukee Blood Center, Inc. *James J. Griffitts, M.D.,* Associate Director, John Elliott Blood Bank of Dade County, Miami, Florida. Presentation of certain aspects of blood bank work and immunihematology; summary of the present state of affairs and possible unsolved problems; a short intermission will be followed by a question period.

2. "Mycobacterium"

Moderator: Mary Turner, M.S., M.T. (ASCP), Bacteriologist in Charge of the Bacteriology Laboratory, University of Illinois Research and Education Hospitals.

Panelists: Five panelists including three technologists, *Ralph Hubble, Ph.D.,* Director of Laboratories of the Chicago-State Tuberculosis Sanitarium and *Sol Roy Rosenthal, M.D., Ph.D.,* Associate Professor of Preventive Medicine, University of Illinois College of Medicine, will discuss various topics. A demonstration and discussion period will follow the talks. The demonstrations will show various types of Mycobacterium on a variety of media.

3. Radioactive Isotopes

Moderator: Dr. Robert J. Hasterlik, Associate Professor of Medicine, University of Chicago School of Medicine, and Associate Director, Argonne Cancer Research Hospital.

Panelists: Dr. John A. Cooper—Associate Professor of Biochemistry and Assistant Dean, Northwestern University Medical School. *Dr. Y. Thomas Oester*—Professor of Pharmacology and Chairman of the Department of Pharmacology, Strich School of Medicine, Loyola University, and Acting Director Radioisotope Laboratory, Hines Hospital. *Dr. Clifford Gurney*—Assistant Professor of Medicine, University of Chicago School of Medicine. *Dr. Ernest Beutler*—Assistant Professor of Medicine, University of Chicago School of Medicine.

These speakers are all actively working in the field of radioisotopes.

SILVER THEATER: Movies will be shown on various subjects. Have you titles of films, or the films themselves which you would like to have featured? Contact the production manager of this theater, Miss Grace Knox, 5731 Blackstone, Chicago 37, Illinois.

MEMORY LANE: Have you snapshots or photographs of by-gone conventions that could be posted on a special bulletin board at the convention? Be sure to write the names of the people on the back of each (faces and figures have a faculty of changing over the years). Send them to Miss Elizabeth O'Connor, 2203 Ridge Avenue, Evanston.

VISITING MEDICAL TECHNOLOGISTS AND LABORATORY TECHNICIANS ARE WELCOME to attend the convention. They must pay the $8.00 registration fee and have a visitor's badge in order to attend any sessions and events scheduled. Since Work Shops and Study Group attendance is limited, members and student members will be assigned first. Any names of those registering in advance for whom assignments cannot be made immediately, will be placed on a waiting list.

HOTEL RESERVATION BLANK

Twenty-fifth Annual Convention of the
American Society of Medical Technologists
June 23-28, 1957
PALMER HOUSE
State and Monroe Streets
Chicago, Illinois

Please reserve for arrival on _____
day date time

Check type of accommodation desired

Single	Double Bed (2 persons)	Twin Beds (2 persons)	Suites—2 Rooms (1 person)	Suites—3 Rooms
☐ $ 7.50	☐ $13.50	☐ $15.50	☐ $30.50 and up	☐ $55.00 and up
☐ $ 8.50	☐ $14.00	☐ $16.00		
☐ $ 8.75	☐ $15.50	☐ $16.50	Additional person $4.00	
☐ $ 9.00	☐ $16.00	☐ $17.00		
☐ $10.00	☐ $17.50	☐ $17.50	Dormitory accommodations	
☐ $11.50	☐ $19.00	☐ $18.00	4 persons to a room $4.00 each	
☐ $12.50		☐ $19.00		
☐ $14.00		☐ $21.00		

If not able to reserve a room at the rate requested, substitute a room at the next available rate.

Confirm Reservation to: NAME _____

ADDRESS _____

CITY _____

STATE _____

Persons sharing the room are listed below

_____

Make Hotel reservations direct with the Palmer House.

SISTERS' HOUSING

Arrangements have been made with the Palmer House to house the Sisters together. This was done in order that as many as possible will be able to stay at the hotel. Please send your reservations to **Sister Hilda Krseminski, St. Francis Hospital, Evanston, Illinois.** Sr. Hilda will then make arrangements for room with the Palmer House. She will also take care of accommodations when convent housing is necessary. Please make your reservations as soon as possible. Be sure to include your date and time of arrival.

ADVANCE REGISTRATION

This is a **must** in the day and age of so many budget-minded people. It will seem so much less expensive if you try to send a little money before hand by registering in advance. What a relief not to have to stand in line after a long journey to the convention.

Save disappointments by signing up for the workshops, study groups and round table luncheons.

First come first served. Do it now—do not ponder. You will not regret your decision. Use the Registration forms on following pages.

ADVANCE REGISTRATION
SILVER ANNIVERSARY CONVENTION
American Society of Medical Technologists
June 23-28, 1957
Palmer House, Chicago, Illinois

Advance registration application and fees will be accepted if mailed on or before May 20, 1957.

Name_____

Address_____

Local Newspaper_____

Number	Event	Price	Total
_____	Registration . . . Member ASMT	$ 5.00	$_____
_____	Technical Non-member	8.00	_____
_____	Student Non-member	5.00	_____
_____	Student Member	none	
_____	Guest of Member	none	
_____	Sunday Reception	none	
_____	Tuesday . . . Edgewater Beach Hotel	8.50	_____
_____	Wednesday . . . Round Table Luncheons	3.50	_____
_____	Thursday . . . Banquet	10.00	_____
_____	Workshops	6.00	_____
_____	Study Groups	3.00	_____
		Total	$_____

IF MORE THAN ONE REGISTRATION, LIST NAMES BELOW

_____

_____

_____

Where a registration fee is required, this MUST accompany the advance registration for other events.

MAKE CHECKS OR MONEY ORDERS PAYABLE TO "ASMT 1957 CONVENTION." MAIL APPLICATION AND FEES TO:

ASMT CONVENTION
25 HERMANN PROFESSIONAL BUILDING
HOUSTON 25, TEXAS

ALL RESERVATIONS FOR ROUND TABLE DISCUSSIONS, WORKSHOPS, STUDY GROUPS, AND OTHER EVENTS MUST BE IN CONJUNCTION WITH ADVANCE REGISTRATION.

ROUND TABLE LUNCHEON: Wednesday, June 25th, 11:30-1:30 P.M., is your chance to question an expert in your choice of subject at a luncheon table at the Palmer House. Conversation and information will be "batted" around the ten at each table. Your advance registration will assure you a place at a specific table to be assigned at the registration desk.

Round table discussion on:

Hematology....	Serology....
Histology....	Chemistry....
Parasitology....	Blood Bank....
Bacteriology....	Mycology....
Urinalysis....	

Please indicate your 1st, 2nd and 3rd choices with numbers.

WORKSHOPS AND STUDY GROUPS

Limited enrollment, Members and Student Members will have priority.

Study Groups To be held in the Palmer House. Fee $3.00. They will consist of demonstrations and lectures.

1. Chemistry—Half-day sessions will be repeated three times.
2. Cytology and Histology—Half-day sessions will be repeated three times. Thursday afternoon for non-delegates only.

Workshops To be held away from the Palmer House. Transportation and material costs will be included in the work shop fee of $6.00. Practice will be provided in technical methods.

1. Mycology—One half-day session.
2. Medical Photography—Half-day sessions will be repeated twice on black and white gross photography. Friday morning limited to non-delegates. One half-day session on photomicrography.
3. Bone Marrow and Hematology—One all day session.
4. Tissue Culture—Morning sessions repeated twice.

Check Study Group or Work Shop reservation desired. No reservation will be accepted unless accompanied by Advance Registration Form and payment in full. If a member is elected a delegate, his schedule may be changed accordingly.

STUDY GROUP 1. Chemistry.....Monday p.m.____
Tuesday a.m.____
Tuesday p.m.____
2. Cytology and Histology.....Wednesday a.m.____
Wednesday p.m.____
Thursday p.m.____

WORK SHOP: 1. Mycology.....Wednesday p.m.____
2. Medical Photography
Gross Black and White....Wednesday p.m.____
(non-delegates only)..Friday a.m.____
Microphotography.....Thursday a.m.____
3. Bone Marrow and Hematology.....Saturday June 29____
4. Tissue Culture.....Thursday a.m.
8:45-10:15____
10:30-12:00____

See the News Release for more information concerning Study Groups and Workshops.

SCIENTIFIC EXHIBIT: Write for application blanks and information if you would like to have a booth assignment for exhibiting your special techniques—something new or a new method. Scientific Exhibits Chairman, #25 Hermann Professional Bldg., Houston 25, Texas, by Apr. 1, 1957.